"Effect of Hormonal and non -hormonal therapy on induction of estrous and serum biochemical profile in anoestrus crossbred cattle"

THESIS

SUBMITTED TO THE

BIHAR AGRICULTURAL UNIVERSITY

(FACULTY OF POST-GRADUATE STUDIES)

SABOUR (BHAGALPUR), B I H A R

In Partial fulfillment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(ANIMAL REPRODUCTION, GYNAECOLOGY & OBSTETRICS)

By

Dr. Chanda Kumari

Regd. No. **M/VOG/47/2008–09**

DEPARTMENT OF ANIMAL REPRODUCTION,GYNAECOLOGY & OBSTETRICS

BIHAR VETERINARY COLLEGE

P A T N A – 800 014

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ABSTRACT

The present study was carried out comprising 30 anoestrus crossbred cows and 10 normal cycling crossbred cows maintained at Composite livestock cattle farm, Bihar veterinary college, Patna; organised and un-organised private Khatals around Patna and cross-bred cows brought at veterinary clinical complex BVC Patna.

The anoestrus crossbred cows were selected on the basis of owner's history and repeated gynaeco-clinical examination of reproductive organs at 10 days intervals. Incorporation of normal cyclic crossbred cows was done on the basis of owner's history, examining colour and consistency of oestrual mucal discharge and per rectal examination of genital organs. All selected animals were dewormed with broad spectrum ecto parasitic drug and categorised into 4 groups.

Main objectives of the present studies were to observe the effect of administration of hormonal(Human placental extract) and non-hormonal(Clomiphene-citrate) products on changes in total protein, total cholesterol, calcium, inorganic phosphorus, magnesium, zinc, manganese, copper and cobalt in blood serum collected on 0 day, 5^{th} day, 10^{th} day, 15^{th} day& 21^{st} day after following of the treatment. Besides that, the effect of both hormonal and non-hormonal products were accessed on induction of oestrus and conception rate in anoestrus crossbred cows. The experiment was carried by maintaining four groups of animals in which two groups i.e. Human placenta extract treated group (T_1), Clomiphene-Citrate treated group (T_2) were kept as treatment groups and two controls groups C_1 and C_2 comprising of anoestrus

and normal cycling crossbred cows respectively. Anoestrus control group (C_1) was utilized to access the comparative efficacy of both hormonal and non-hormonal drugs on induction of oestrus as well as post-treatment comparative variation in the level of different biochemicals profile in comparison of treatment groups. Besides that, the normal cycling crossbred cows(C_2) were utilized for accessing variation in blood biochemicals profile viz. serum total protein, total cholesterol, serum calcium, serum inorganic phosphorus, serum magnesium, serum zinc, serum manganese, serum copper, serum cobalt in comparison of anoestrus crossbred cows on different durations of blood collection.

The mean value of serum total protein in placentrex treated group (T_1) showed an increasing trend and were found to be 6.77±0.07 and 7.38±0.10gm% in blood serum samples collected at 0 day and 21st day of collection respectively. The level of serum total protein in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 6.84±0.09 and 7.48±0.94gm% at 0 day and 21st day respectively. The corresponding values of serum total protein in untreated anoestrus cows (C_1) were 6.87±0.24 to 6.90±0.24gm% at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in both T_1 and T_2 groups showed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups showed the non-significant variation within themselves though, significant increase in the level of serum total protein were recorded at different days of collection from 0 to 21st day in both treatment groups.

The mean value of total protein in anoestrous crossbred cow (C_1) was found to be varied from 6.87±0.24 to 6.90±0.24 (gm %) in collected blood serum samples at 0 and 21st day of collection but its variations were found non-

significant. The corresponding mean value of serum protein in normal cyclic crossbred cows (C_2)were also showed non-significant variation from 7.46±0.08gm% at 0day of collection to 7.49±0.08gm% at 21st day of collection. Comparison in the level of total serum protein between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant ($P\leq 0.01$) variations.

The mean value of cholesterol in placentrex treated group (T_1) showed an increasing trend and were found 143.32±2.38mg% and 168.85±2.23mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum cholesterol in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 143.28±4.05 mg% and 159.75±2.48 mg% at 0 and 21st day of collection. The corresponding values of total serum cholesterol in untreated anoestrus cows (C_1) ranged from 142.41±1.56mg% at 0 day of collection to 146.10±1.31mg% at 21st days of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group respectively showed significant ($P\leq 0.01$) variation in both the treatment groups.

. Mean value of serum cholesterol in anoestrous crossbred cow (C_1) was found to be varied 142.41±1.56 and 146.10± 1.31(mg %) in collected blood serum samples at 0 and 21st day of collection but these variations were found non-significant. The mean value of serum cholesterol in normal cyclic crossbred cows (C_2) also showed non-significant variation from 167.78±1.27 at 0 day of collection to170.25±1.25 at 21st day of collection. Comparison in the level of total cholesterol between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant ($P\leq 0.01$) variations.

The mean value of serum calcium in placentrex treated group (T_1) showed an increasing trend and were 9.69±0.26 and 10.83±0.26mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum calcium in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 9.58±0.21and10.88±0.21mg% at 0 and 21st day of blood collection. The values of serum calcium in untreated anoestrus cows (C_1) ranged from 9.65±0.11mg% at 0 day to 9.75±0.11mg% 21st day of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group showed significant ($P\leq0.01$) variation in both the treatment groups. However, comparison in the level of calcium between these treatment groups showed non-significant variation within themselves though, significant increase in the level of serum calcium were recorded at different days of collection from 0 to 21st day in both treatment groups. Comparison in the level of serum calcium between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P\leq0.01$) variations.

The mean value of serum inorganic phosphorus in placentrex treated group (T_1) showed an increasing trend and were 3.15±0.03mg% and 3.57±0.01mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum inorganic phosphorus in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 3.17±0.03mg% and 3.46±0.02mg% at 0 and 21st day respectively. The level of serum inorganic phosphorus in untreated anoestrus cows (C_1) showed little variation and ranged from 3.12±0.02mg% at 0 day of collection to 3.22±0.02mg% at 21st day of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group showed significant ($P\leq0.01$) variation in both the

treatment groups and among different periods of blood collection. However, comparison in efficacy of those hormonal and non-hormonal drugs between these treatment groups showed the non-significant variation within themselves at 0 day and 10^{th} day of collection whereas, significant($P \leq 0.01$) variation in the level of inorganic phosphorus was recorded at 5^{th} ,15^{th} ,21^{st} day of collection.

The mean value of serum inorganic phosphorus in anoestrous crossbred cow (C_1) was found to be varied from3.12±0.02 to 3.22± 0.02(mg %) in collected blood serum samples at 0 and 21^{st} day of collection but its variations were found to be non-significant. The mean values of serum inorganic phosphorus in normal cyclic crossbred cows (C_2) also showed non-significant variation from3.48±0.01 at 0 day of collection to 3.54±0.01 at 21^{st} day of collection. Comparison in the level of serum inorganic phosphorus between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P \leq 0.01$) variations.

The mean value of serum Magnesium in placentrex treated group (T_1) showed an increasing trend and were 2.05±0.20 and 2.76±0.21mg% in blood serum samples collected at 0 day and 21^{st} day of collections respectively. The level of serum Mg in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 2.04±0.20 and 2.75±0.13mg% at 0 and 21^{st} day respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group respectively revealed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups showed the non-significant variation within themselves though, significant increase in the level of serum Magnesium were recorded at different duration of collection from 0 to 21^{st} day. Comparison in the level of serum

magnesium between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P \leq 0.01$) variations.

The mean value of serum Zinc in placentrex treated group (T_1) showed an increasing trend and were found to be 0.89±0.08 and 1.63±0.08 (PPM) in blood serum samples collected at from 0 day and 21st day of collection. The level of serum Zinc in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.87±0.08 (PPM) and1.66±0.07 (PPM) at 0 and 21st day respectively. The corresponding values of serum Zinc in untreated anoestrus cows (C_1) were 0.89±0.01 (PPM) at 0 day of collection and 0.89±0.01 (PPM) at 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups showed significant ($P \leq 0.01$) variation in both the treatment groups. However, duration wise comparison within these treatment groups showed the non-significant variation within themselves at 0 day,10th 15th, 21st day of collection but significant($P \leq 0.01$) variation was recorded in blood samples collected at 5th day of collection. Comparison in the level of serum Zinc between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P \leq 0.01$) variations.

The mean value of serum Manganese in placentrex treated group (T_1) showed an increasing trend and were found to be 0.96±0.13 and 1.56±0.11 (PPM) in blood serum samples collected at 0 day and 21st day of collection respectively. The level of serum Manganese in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.92±0.10 at 0 day and 1.53±0.09(PPM) 21st day of collection. The corresponding values of serum Manganese in untreated anoestrus cows (C_1) were 0.94±0.03 and 0.95±0.03 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group revealed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups at various stages of collection showed non-significant variation within themselves though, significant increase in the level of serum Manganese were recorded at different days of collection from 0 to 21st day of collection in both treatment group.

The mean value of serum Manganese in anoestrous crossbred cow (C_1) was found to be varied from 0.94±0.03 and 0.95± 0.03(PPM) in collected blood serum samples at 0 and 21st day of collection but its variations were found non-significant. The corresponding mean value of serum Manganese in normal cycling crossbred cows (C_2) were also showed non-significant variation from 1.38±0.13 (PPM) at 0 day of collection to 1.49±0.12 (PPM) at 21st day of collection. Comparison in the level of serum Manganese between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant ($P \leq 0.01$) variations.

The mean value of serum Copper in placentrex treated group (T_1) showed an increasing trend and were found to be 1.31±0.04 and 1.70±0.07 (PPM) in blood serum samples collected at 0 day and 21st day of collection. The level of serum Copper in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 1.29±0.03and1.68±0.04 (PPM) at 0 and 21st day respectively. The corresponding values of serum Cu in untreated anoestrus cows (C_1) were 1.30±0.04 and 1.35±0.04 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups showed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups

showed the non-significant variation at different stages of collection within themselves though, significant increase in the level of serum Copper were recorded at different days of collection from 0 to 21st day in both treatment groups.

The mean value of serum Cobalt in placentrex treated group (T_1) showed an increasing trend and were found to be 0.51±0.01 (PPM) and 0.88±0.03 (PPM) in blood serum samples collected at 0 day and 21st day of collection. The level of serum Cobalt in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.53±0.01 (PPM) and0.83±0.04 (PPM) at 0 and 21st day respectively. The corresponding values of serum cobalt in untreated anoestrus cows (C_1) were 0.52±0.02 to 0.54±0.01 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups respectively showed significant ($P\leq0.01$) variation in both the treatment groups.

The mean value of serum Cobalt in anoestrous animal (C_1) was found to be varied and recorded as 0.52±0.02 and 0.54± 0.01(PPM) in blood serum samples at 0 and 21st day of collection but its variations were found non-significant. The corresponding mean value of serum Cobalt in normal cyclic crossbred cow (C_2) was found to be varied non-significantly and were recorded as 0.61±0.01and 0.63±0.01(PPM) at 0 day and 21st day of collection respectively. Comparison in the level of serum cobalt between anoestrus crossbred cows (C_1) and normal cyclic crossbred cow (C_2) showed significant ($P\leq0.01$) variations.

Based on findings, recorded in the present studies it could be concluded that the administration of both hormonal(Human placental extract) and non hormonal(Clomiphene-citrate) drugs used significant($P\leq0.01$) increase in post

treated blood serum concentration of total protein, cholesterol, calcium, inorganic phosphorus, Magnesium, Zinc, Manganese,Copper,Cobalt.Which might had improved the hypophyseal gonadal functions and ultimately improved ovarian follicular activity resulting in initiation of oestrus. The increased level of total protein and different in ionic concentrations Viz.Inorganic phosphorus,Magnesium,Copper and Zinc observed after administration of those two types of drug were postulated to be involved in enhancing metabolic activity and creating favourable uterine environment besides stimulation of pituitary ovarian axis, resulting ovulation and increased percentage of conception.

Comparison in efficacy of induction of oestrus and the percentage of conception between those two utilized drugs showed 20.0% higher induction of oestrus and 17.46% increased conception rate after administration of placental extract in comparison of clomiphene-citrate treated group in which induction of oestrus and conception rate were found 90% and 88.88% respectively. Thus, it was concluded that administration of placental extract would be more effective for induction of oestrus and increasing of conception rate in anoestrus crossbred cows in climatic condition of Bihar. However, further detailed investigation need to be conducted on larger population to explain the mechanism of varying biochemical profiles and made of enhancing reproductive physiology by administration of those utilized hormonal and non-hormonal drugs.

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

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Dr. R.P. Pandey
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CERTIFICATE-I

This is to certify that the thesis entitled **"Effect of Hormonal and non- hormonal therapy on induction of estrous and serum biochemical profile in anoestrus crossbred cattle"** submitted in partial fulfillment of the requirements for the award of degree of Master of Veterinary Science **(Animal Reproduction, Gynaecology & Obstetrics)** of the Faculty of Post-Graduate Studies, Bihar Agriculture University, Bihar, Sabour, Bhagalpur, is the record of bonafide research work carried out by **Dr. Chanda Kumari, Registration No.: M/VOG/47/2008–09** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

This is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

(Dr.R.P.Pandey)
Major Advisor

Endorsed

(Chairman/ Head of Department)

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BIHAR VETERINARY COLLEGE, PATNA- 800014
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CERTIFICATE-II

We, the undersigned members of the advisory committee of **Dr. Chanda Kumari, Registration No.: M/VOG/47/2008-09,** a candidate for the degree of **Master of Veterinary Science** with major in **Animal Reproduction, Gynaecology & Obstetrics**, have gone through the manuscript of the thesis and agree with the thesis **"Effect of Hormonal and non- hormonal therapy on induction of estrous and serum biochemical profile in anoestrus crossbred cattle"** It may be submitted by **Dr. Chanda Kumari** in partial fulfillment of the requirements for the degree.

(Dr.R.P.Pandey)
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DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

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CERTIFICATE-III

This is to certify that the thesis entitled **"Effect of Hormonal and non- hormonal therapy on induction of estrous and serum biochemical profile in anoestrus crossbred cattle"** submitted by **Dr. Chanda Kumari, Registration No.: M/VOG/47/2008-09** in partial fulfillment of the requirements for the Degree of Master of Veterinary Science **(Animal Reproduction, Gynaecology & Obstetrics)** of the **Faculty of Post-Graduate Studies, Bihar Agriculture University, Bihar, Sabour, Bhagalpur,** was examined and approved on ...../2012.

(Dr R.P.Pandey)
Chairman, Advisory Committee **External Examiner**

Members of Advisory / Examination Committee:

1) **Dr. S.K. Choudhary.**

2) **Dr. K.G. Mandal**

3) **Dr. S. Samantaray**
(Nominee of Dean, Post-Graduate Studies)

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1 INTRODUCTION

Livestock sector plays an important role in providing nutrition, food (milk, meat and egg), skin, hide, wool and organic manure besides providing of draught power, essential for agriculture farming. Nearly 70 percent of rural families owning livestock are small marginal and landless farmers in which livestock rearing is the mainstay of their family income and employment. The strength of the Indian dairy lies in the fact that despite limited resources and investment, it has shown consistent growth. At present the total population of cattle in India is 199.08 million (Livestock census 2007).

Dairy development in India has been acknowledged one of the successful developmental programme as the contribution of milk alone to the national economy (Rs. 82.628 cores) is higher than paddy (Rs. 68,230 cores), wheat (Rs. 40,323 cores), and sugarcane (Rs 23,314 cores). Though India ranks second in cattle population its position in milk production stand at the top.

The present, contribution of agriculture and allied sectors is about 13.8% of its overall national GDP. Moreover, its growth rate is about 2.1%. It is highly interesting that growth of animal husbandry sector is about 4.1% and its total contribution to overall GDP is 5.4% which is about 32% of the total agricultural sector. Generally, agriculture is not proving profitable to crop farmers in the state, nevertheless, livestock, poultry and fishery production has increased their contribution to GDP from 10% to 26% in the last decade through amplifying growth trends to 7% and that of employment to 9% in the state. The livestock and related economic activities have become progressively more profitable to

farmers and in reducing the malnutrition especially among the women and children.

Anoestrus comprises about 2/3rd of infertility problems and thus forms a major condition occurring in crossbred cattle (Kutty & Ramchandran 2003). The incidence of anoestrus in dairy cattle is attributed to the causes of defective managerial practices in the farms and involves genetic factors which ultimately influence upon the economy of production (Roberts, 1971; Boyd, 1977; Roche *et al.*1992). True anoestrus condition develops due to temporary suppression of optimum endocrine secretion to activate the ovarian folliculogenesis for optimum recruitment of dominant follicle.

Anoestrus is multifactorial syndrome which includes the factors like age, breed, environment, nutritional status, managemental practice and general diseased condition, which influences the reproductive performance and as a result there may be delay in puberty in heifers and prolongation in the post-partum anoestrus conditions in cow. It has been observed that balance level of nutrition reduces the age and obtains normal body weight at puberty (Sorensen *et. al.*, 1959; Bosticco and Corrias, 1960; Kopeeky *et. al*., 1973; Zaoral *et. al.*, 1972; Mathai and Raja, 1976 and Gujar and Shukla, 1990).

The occurrence of first post partum estrus is dependent on a number of factors such as involution of uterus, endocrine constitution, hormonal balance, nutritional status, uterine health, season, parity, suckling & others (Arthur *et. al.*, 1982). Nutritional deficiencies or excess of nutritional supply are frequently referred to as the cause of infertility (Parker and Blowey, 1976 & Francos *et. al.*, 1977), Nutritional stresses can effect the reproductive system and may be manifested as delayed return to cyclicity and failure to become pregnant. Successful establishment of pregnancy requires a series of coordinated signals involving the brain, ovary and uterus all of which may be influenced

nutritionally at the macro or micro level. Efficient production in domestic animals requires that the essential nutrients in diet be provided in appropriate amounts and in forms that are most biologically useful (Boland, 2003). Protein deficiency may delay onset of puberty and hamper post-partum reproductive performance due to reduced gonadotropin release and decrease anterior pituitary responsiveness to GnRH.Deficiency of protein also suppresses the synthesis and secretion of LH but not the secretary activities of FSH cells. Protein deficiency as well as its excessive intake causes acyclia and low conception rates. Cholesterol being precursor of steroid hormone, influence the reproductive functions like oestrus, pregnancy, lactation etc. (Purohit and Kohli, 1977). Total plasma protein level in animal blood has been considered to be the direct reflection of metabolic turnover of the body.

Minerals play an important role in the regulation of reproduction and production in animals through their involvement in certain enzyme system. About one half of the total plasma calcium available as Ca^{+} is physiologically active. Similarly, inorganic phosphorous is practically found entirely in the plasma. Both the elements are essential for bone formation and its deficiency lead to acyclicity. Magnesium is equally essential in all enzyme relations catalyzed by ATP and in maintaining the physical integrity of RNA – DNA.

Trace elements viz. Zn, Fe, Cu, Co & Mn are the integral parts of many metalloenzymes and carrier of proteins, function as Co-structure, as activatory of enzymes or stabilizers of secondary molecular structure (Valee & Wacker, 1976) and play an important role in body metabolism, protein synthesis, immunocompetence and even in the maintenance of vascular and skeletal integrity and structure and function of the central nervous system (Hidiroglou, 1979). The imbalance or deficiency of trace elements leads to inactive ovaries and repeat breeding in dairy animals.

Copper and low molecular weight copper complexes have angiogenic property (Keen *et. al*., 1998). It thus may help in vessel integrity and vasculature in the genital organs and in the developing fetus. Cytochrome oxidase is a cupro-enzyme necessary for electron transport in mitochondria for energy metabolism of ATP dependent biosynthesis reactions (eg. Phospholipids required for neural myelination).

Copper deficiency leading to impaired reproduction is hampered in a manner of depressed estrous. Changes in steroidal metabolism may lead to alter of reproductive behaviour showing nymphomania in ewes (Hidiroglou, 1979). Pasture of low copper content has high incidence of infertility on the grazing herd which is associated with anaemia Kreplin and Yaremico (1992) listed that copper deficiency is associated with delayed puberty, poor fertility retained placenta and repeat breeding besides reduced conception rate and increased days open .

Cobalt has been found to be required in the synthesis of Vit. B_{12} & its deficiency has been associated with anoestrus, delay onset of puberty (Hidiroglou, 1979: Pugh *et. al.,* 1985), abortions, nonfunctional ovaries (Wagner, 1962), birth of weak calves and general infertility (Alderman, 1963). It has been observed by several workers that cobalt deficiency impairs breeding performance and the most common manifestation of cobalt deficiency is marked reduction in conception rate with reduction in duration of oestrus during normal breeding season.

Manganese (Mn) is involved in activities of several enzyme systems including hydrolases, kinases, decarboxylases as well as Fe containing enzymes. Mn is an important Co-factor for glycosyl transferase, Galactotransferase & Pyruvate carboxylase. Mn function in synthesis of sterols can also be correlated with the synthesis of Gonadal steroid hormones. The pituitary are relatively

rich in Mn and these organs are sensitive to its deficiency in diet (Hidiroglou, 1979). Increase uptake of Mn during 4-11 days of estrus cycle also suggests that it specifically involved in luteal metabolism. Reproductive performance may be altered by absent or irregular estrous cycle, decreased conception rate, higher abortion cases and low birth weight and weak calves with deformed legs are observed in manganese deficiency (Hidiroglou, 1979; Kreplin, 1992). Poor follicular developments with delayed ovulation, increase in service per conception and embryonic mortality are also listed as the adverse effect of manganese deficiency.

Zn is a component of several metalloenzymes and thus activates several enzyme systems which in turn play a vital role in hormone secretion, especially related to growth, reproduction, immunocompetence & stress. It is a component of Cu-Zn superoxide dismutase, Carbonicanhydrase, alcohol dehydrogenase, alkalinephosphatase & RNA polymerase. In female, Zn deficiencies have been associated with abortion, fetal mummification, lower birth weights, and prolonged labour as zinc plays important role in uterine lining.

Zn deficiency may cause delayed puberty, lower conception rate (kreplin 1992), failure of implantation and reduction of litter size (Hidiroglou, 1979).

Anoestrus due to hormonal imbalance is also an important cause of infertility in cow. In anoestrus animals there are inadequate hypothalamic stimuli for the release of gonadotropin (Shokier, 1959).

Hence, it is assumed that blood biochemical and mineral profiles during post- partum period have great relevance to future fertility in dairy animals and so, studies of these factors in blood of post- partum cows may be a potential aid in characterizing and the diagnosis of mineral related disorders in post parturient cows.

Human placental extract is a biological source of three releasing hormones viz. GnRH, TSH – RH and CRF (Gibbons *et al.* 1975; Lee *et al.* 1981; Khodr & Siler – Khodr, 1978; Khodr and Siler – Khodr 1980; Shibasaki *et al.* 1982) and its role has been documented in stimulating the follicular growth in true anoestrus condition.

The introduction of clomiphene-citrate in the veterinary profession has been reported to be a good tool for treating anoestrous cows and buffaloes by various workers. Deshpande *et. al*., (1976), Kaikini *et. al*., (1977), Hukeri *et. al*., (1979), Dugwekar *et. al*.,(1980) and Reddy *et. al*., (1990) and in sheep and goat by Sinha *et. al*., (1980) and Sharma *et. al.*, (1983).

Clomiphene-citrate is a non-hormonal combination of two isomers of triphyenylethyl compound containing cis-clomiphene & trans-clomiphene. Each 300mg dose containing cis-clomiphene citrate – 180mg and trans-clomiphene citrate – 120mg. It has been reported that clomiphen-citrate stimulate the hypothalmopituitary axis to release GnRH. Which then activate pituitary gland for secretion of gonadotropin. Reddy *et. al.*, (1990) had observed the induction of 60 % ovulatory estrus in anoestrus cross-bred cow following treatment with clomiphene-citrate.

In a study Khasatiya *et. al.*, (2005) observed that the exhibition of estrum in true anestrous buffaloes after GnRH treatment was recorded 88.33% with a mean interval of 19.33±7.89 days. He further observed that the level of total protein, total cholesterol, calcium, and inorganic phosphorus were significantly lower in true anoestrous than the sub estrus buffaloes is of cyclically functional ovaries. The level of Mg (3.67±0.08), and trace elements (Zn, Cu, Ca, and Mn) however, did not vary between them.

In light of the above recorded facts / observations the present study has been designed to carry research entitled **"*Effect of hormonal & non- hormonal***

***therapy on induction of estrous and serum biochemical profile in anoestrus crossbred cattle*.”** with the following objective –

1. Comparative studies on serum biochemical profile (Cholesterol, total protein, Ca, P, Cu, Co, Zn and Mn) between anoestrus & normal cycling crossbred cattle.

2. To study the influence of human placental extract on induction of estrus and serum biochemical profile in anoestrus crossbred cattle.

3. To study the influence of Clomiphene - citrate on induction of estrus and serum biochemical profile in anoestrus crossbred cattle.

4. To study the efficacy of human placental extract and Clomiphene – citrate on conception.

3 MATERIALS AND METHODS

Experimental Animals:

The present study was carried out on 30 anoestrus crossbred cows and 10 normal cycling cows maintained at Composite livestock cattle farm, Bihar veterinary college, Patna, organised and un-organised private Khatals around Patna and cross-bred cows brought at veterinary clinical complex BVC Patna.

The anoestrus crossbred cows were selected on the basis of owner's history and repeated gynaeco-clinical examination of reproductive organs at 10 days intervals. Incorporation of normal cyclic crossbred cows were done on the basis of owner's history, examining colour and consistency of oestrual mucus discharge and per rectal examination of genital organs. All selected animals were dewormed with broad spectrum ecto parasitic drug and categorised into 4 groups.

TREATMENT GROUP:

Group I (T_1): Comprising of 10anoestrus crossbred cows treated with human placental extract at dose rate of (15 ml I/V) for 3 consecutive days.

Group II (T_2): Comprising of 10 anoestrus crossbred cows treated with clomphene- citrate@ 300 mg daily for 5 days.

CONTROL GROUP:

Group III (C_1): Comprising of 10 anoestrus crossbred cows in which no treatment was followed and were utilized to compare the blood biochemical profile with normal cyclic crossbred cows.

Group IV (C_2): Consists of 10 normal cyclic crossbred cows.

Deworming:

The cows, utilized for the present studies were dewormed with broad spectrum anthelminthic drug fenbendazole @ 3 gm/300 kg body weight.

TREATMENT:

GROUP WISE TREATMENT SCHEDULE

Group of animal	**T_1**	**T_2**	**C_1** (Anoestrus control)	**C_2** (Normal cycling)
No. of Animal	10	10	10	10
Drug to be used	Human placental extract	Clomiphene citrate	Normal saline	Nil
Dose rate	@ 10 ml in heifers & @ 15 ml at 24 hr interval for 3 days.	@ 300mg daily for 5 days.	@ 10 ml daily for 3 days.	Nil
Route	I/V	Orally	I/V	Nil

Composition of drug under trial:

Name of Drug	Composition
Placentrex* (Human placental extract)	Each ml of placentrex is derived from 0.1gm of fresh human Placenta. Total nitrogen not more than 0.08% w/v Benzyl Alcohal IP 1985, 1.5 % v/v (as preservative) water for injection I.P. q.s.
Ovulanta Kit** (Clomiphene citrate)	Each clomiphene citrate (300mg) bolus containing cis-clomiphene citrate – 180mg and trans-clomiphene – 120mg.

*Placentrex - Albert Devid Limited, Kolkata-50.

*Ovulanta-Kit -Vetmankind Division of Mankind pharma ltd. New Delhi 110020

Collection of blood samples and its preservation:

The blood samples from each cow were collected in a sensible manner. Attempts were made to avoid a violent effect on them. Blood samples were collected directly from jugular vein through vein puncture by sterilized hypodermic needle (18 gauge) and disposable syringe (20 ml). Approximately 15ml. blood from each cow was collected and kept in glass test tube in slanting position for half an hour at room temperature for separation of serum. The serum was separated and centrifuged for 10 minutes at 3000 rpm.

Then clean serum was pipetted out with the help of sterilized Pasture pipette and kept in 10 ml stopper clean vial. The separated serum was kept in refrigerator at -20°C till further analysis.

Scheduled of blood sampling:

Blood samples were collected from Anoestrus non-treated cows, normal cyclic and anoestrus treated cows at following interval schedule.

- Blood samples were collected on 0 day at the start of treatment schedules.
- Blood samples were again taken on **5th** days from start of the treatment schedules.
- Blood samples were then collected on **10th** day from start of the treatment schedules.
- Blood was collected on **15th** day at the start of the treatment schedules.
- Blood was collected on **21st** days at the starts of treatment schedules.

Analysis of blood samples:

The collected blood samples were processed for estimation of biochemical profiles. The estimation of Total protein, cholesterol, calcium, phosphorus, Magnesium, Manganese Copper, Cobalt, and Zinc were done in serum.

Determination of Total serum protein:

Estimation of total protein was carried following the **Biuret method** utilizing Total protein test kit supplied by Span diagnostic limited, Gujrat.

Reagents used:

Reagent No.	Reagent	Composition	Concentration
1.	Biuret reagent	Copper sulphate Sodium Hydroxide Sodium-Potassium Tartrate Surfactant	7mM/L 200mM/L 20mM/L qs
2.	Protein Standard	BSA Preservative	6.5 g/dl qs

The reagents were ready to use.

Assay principle:

The peptide bonds of protein react with cupric ions in alkaline solution to form a coloured chelate, the absorbance of which is measured at 578nm. The biruet reagent contains sodium-potassium tartrate, which helps in maintaining solubility of this complex at alkaline pH. The absorbance of final colour is proportional to the concentration of total protein in the sample.

Procedure:

The serum sample and the reagents were mixed in clean dry test tube and labelled as Blank (B), Standard (S) and Test (T) in the proportions given below.

Pipette into tube marked	Blank (B)	Standard (S)	Test (T)
Serum	----	----	10µL
Reagent 2	----	10 µL	----
Reagent 1	1000 µL	1000 µL	1000 µL

All the test tubes contents were mixed well by shaking and incubated at 37°C for 5 minutes. Programmed the Spectrophotometer as per assay parameters. Absorbance of the standard and each test at 578nm against reagent blank were recorded. Result calculated as per the given calculation formula.

Calculation formula:

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 6.5$$

Determination of Cholesterol was carried following the Enzymatic CHOD-PAP method utilizing Cholesterol test kit supplied by Span diagnostic limited, Gujrat.

Reagents used:

Reagent No.	Reagent	Composition	Concentration
1.	Cholesterol reagent	Good's Buffer (pH 6.7) Cholesterol Esterase Cholesterol Oxidase Peroxidase 4-Aminoantipyrine Stablisers	50mmol/L ≥ 100U/L ≥ 50U/L ≥ 3KU/L 0.4 mmol/L qs
2.	Cholesterol Standard	Cholesterol Preservative Stabliser	200mg/dl qs qs

Assay principle:

Cholesterol esters are hydrolysed by Cholesterol Esterase (CE) to give free Cholesterol and Fatty acids. In subsequent reaction, Cholesterol Oxidase (CHOD) oxidises the 3-OH group of free Cholesterol to liberate Cholest-4-en3-one and Hydrogen peroxide. In presence of peroxidase (POD), Hydrogen peroxide couple with 4-Aminoantipyrine (4-AAP) and phenol to produce Red Quinonemine

dye. Absorbance of coloured dye is measured at 505nm and is proportional to amount of Total Cholesterol concentration in the Sample.

Procedure:

The serum sample and the reagents were mixed in clean dry test tube and labelled as Blank (B), Standard (S) and Test (T) in the proportions given below.

Pipette into tube marked	Blank (B)	Standard (S)	Test (T)
Serum	----	----	10µL
Reagent 2	----	10 µL	----
Reagent 1	1000 µL	1000 µL	1000 µL

All the test tubes contents were mixed well by shaking and incubated at 37°C for 5 minutes. Programmed the Spectrophotometer as per assay parameters. Absorbance of the standard and each test at 505nm against reagent blank was recorded. Result calculated as per the given calculation formula.

Calculation formula:

$$\text{Total Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Determination of ionic concentration:

Calcium, Phosphorus, Copper, cobalt, zinc and manganese were estimated by the **Atomic absorption spectrophotometer (Perkin elmer, A Analyst 200)** at Rajendra Agricultural University, Pusa (Samastipur).

Digestion of Serum:

0.5 ml serum was taken in 100ml conical flask and equal volume of concentrated HNO_3 were kept for overnight. Now, 10-15 ml of diacid mixture of HNO3 and HCLO4 in a ratio of 4:1 were added and kept on a hot plate and heat gently at first. Then the solution heated was vigoursly until solution became clear and white fume ceases to comeout.Heating of solution was discontinue volume is reduced to 1 – 2 ml then it was Cooled and transferred to 50 ml volumetric flask and volume was made up to the mark adding distilled water. Now, it was filtered through Whatman No. 01 filter paper and used for further analysis through Atomic absorption spectrophotometer.

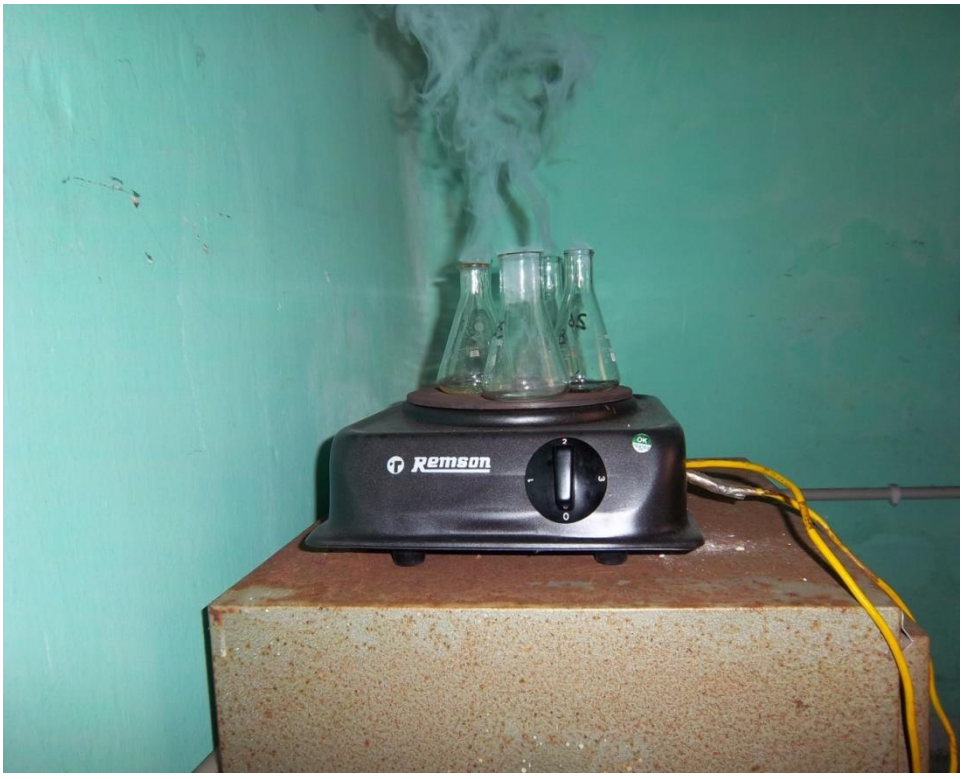
Statistical Analysis:

Statistically the data were analysed to the method suggested by Snedecor and Cocharn (2003).

Photograph No. 1: Collection of blood sample.

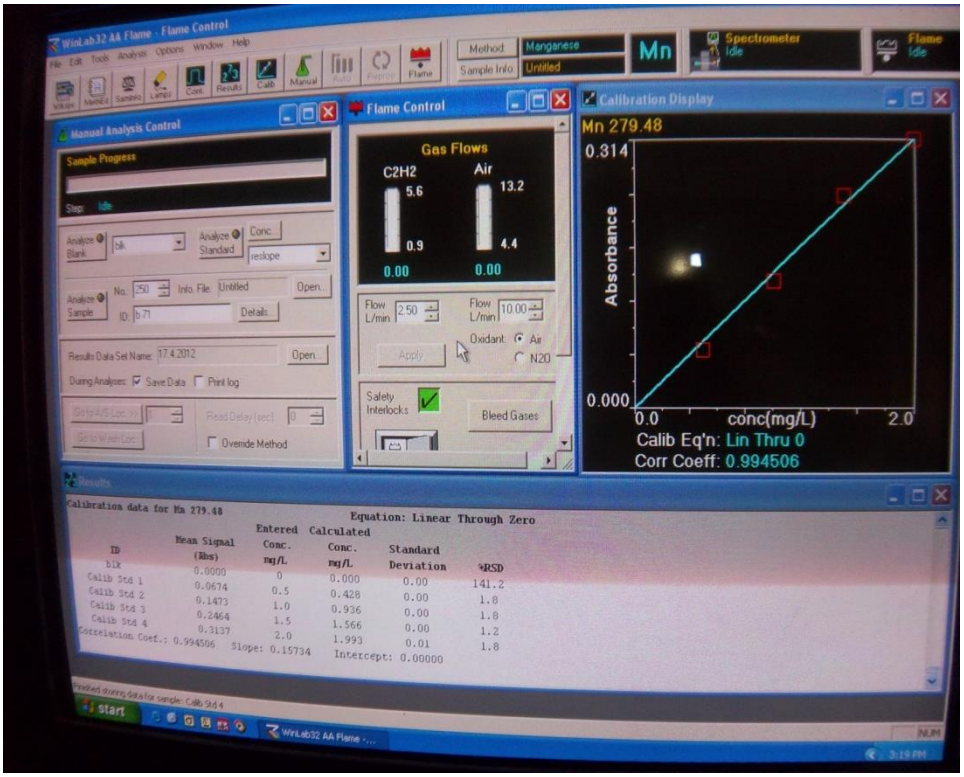
Photograph No. 2: Centrifugation of serum sample.

Photograph No. 3: Digestion of serum sample.



Photograph No. 4: Fumigation of serum sample.

Photograph No. 5: Atomic absorption spectrophotometer.



Photograph No. 6: Reading of serum sample in Atomic absorption spectrophotometer.

4 RESULTS

The study was conducted on 30 anoestrus and 10 normal cycling cross-bred cattle, maintained at organized private khatals, un-organised cattle shed composite livestock cattle farm and animals brought at veterinary clinical complex Bihar veterinary college, Patna. Out of selected 30 anoestrus cows 20 anoestrus cross-bred cows were kept in two groups (T_1&T_2) and treated with human placental extract & clomiphene-citrate respectively. Rest 10 anoestrus cross-bred cows were kept as control in which no treatment was followed. Besides that 10 regular normal cycle cows were utilized for comparing only biochemical profile in comparision of anoestrus cows (C_1) and kept as control (C_2).

Blood samples were collected from the each cross-bred cow of every group at five days interval i.e. on 0, 5^{th} ,10^{th} ,15^{th} and 21^{st} day from the initiation of treatment schedule for study of comparative biochemical variation on administration of both hormonal and non-hormonal drugs.

BLOOD BIOCHEMICAL PROFILE:

Serum Total Protein:

The mean ± S.E. of serum total protein in various group have been presented in Table (1) and fig (1).

The level of serum total protein(gm%) in collected blood of C_1 group at 0 day 5^{th} day 10^{th} day, 15^{th} day , 21^{st} day were 6.87 ± 0.24, 6.87 ± 0.23, 6.87 ± 0.24, 6.88 ± 0.24,6.90 ± 0.24 respectively. The level of serum total protein(gm%) in C_2 group were 7.46 ± 0.08,7.48±0.08,7.48±0.08, 7.48±0.08, 7.49±0.08 at 0 day,5^{th} day,10^{th} day,15^{th} day, 21^{st} day blood serum collection respectively. The level of serum total protein in T_1 group were 6.77± 0.07, 7.05±0.08, 7.27± 0.08,

7.30±0.09, 7.38± 0.10 and in T_2 group were 6.84±0.09, 7.07±0.10, 7.44± 0.09, 7.45±0.09,7.48±0.94(gm%) at 0day ,5th day 10th day ,15th day ,21st day blood serum collection respectively.

Analysis of variance (Table - 2) revealed significant variation between treatments but non-significant effect among periods of blood collection.

The mean values of serum total protein (gm %) in serum samples were found to be 6.87±0.24 and 7.46 ± 0.08 in C_1&C_2 respectively in samples of zero day collection. The mean serum total protein of C_1, T_1, & T_2 groups showed significantly (P≤ 0.01) lowered value by 0.59, 0.69, and 0.62 from the normal cyclic control group (C_2). However, the mean serum total protein value did not differ significantly between anoestrous control group (C_1) and treated T_1 &T_2 groups in blood serum samples at 0 day of collection. The level of total serum protein at zero day collection did not vary significantly between both treatments groups T_1 & T_2.

On 5th day collection, the mean value of serum total protein (gm %) in serum samples were found to be 6.87± 0.23 and 7.48± 0.08 in C_1&C_2 respectively. The mean serum total protein value of C_1 & T_1 group showed significantly (P ≤ 0.01) lowered value by 0.61 and 0.43 from the normal cyclic control group (C_2) but T_2 group did not differ significantly from the normal cyclic control group (C_2). Further, it was observed that the mean serum total protein value of T_1 and T_2 group showed significantly(P≤0.01) higher value by 0.18 and 0.20 in comparison to anoestrous control group (C_1).The level of total serum protein in 5th day collection did not show significant variation between both treatment groups T_1 & T_2.

The trend of 10th day collection was similar to that of 5th day collection. The mean value of serum total protein (gm %) in serum samples were 6.87± 0.24 and 7.48± 0.08 in C_1&C_2 respectively. The mean serum total protein value of C_1 & T_1 group showed significantly (P ≤ 0.01) lowered value by 0.61 and

0.21 from the normal cyclic control group (C_2) whereas; T_2 group did not differ significantly from the normal cyclic control group (C_2). However, the mean serum total protein value of T_1 and T_2 group showed significantly ($P \leq 0.01$) higher value by 0.40 & 0.57 from anoestrous control group (C_1). The level of total serum protein in 10th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 15th day of collection, the mean value of serum total protein (gm %) in serum samples were 6.88± 0.24 and 7.48 ±0.08 in C_1&C_2 respectively. The mean serum total protein value of C_1 showed significantly ($P \leq 0.01$) lowered value by 0.60 from the normal cyclic control group (C_2) but T_1&T_2 did not differ significantly from the normal cyclic control group (C_2). However, the mean serum total protein value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.42 & 0.57 from anoestrous control group (C_1).The level of total serum protein in blood samples of 15th day collection did not show significant variation between both treatments groups T_1 & T_2.

The level of total protein of 21st day collection was almost similar to that of 15th day collection. The mean value of serum total protein (gm %) in serum samples was found to be 6.90±0.24 and 7.49±0.08 in C_1&C_2 respectively. The mean serum total protein value of C_1 showed significantly ($P \leq 0.01$) lowered value by 0.59 from the normal cyclic control group (C_2) but T_1&T_2 did not differ significantly from normal cyclic control group. However, the mean serum total protein value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.48 & 0.58 from anoestrous control group (C_1).The level of total serum protein in blood samples of 21st day collection did not show significant variation between both treatments groups T_1 & T_2.

Total Serum Cholesterol:

The mean ± S.E. of total serum Cholesterol in various group have been presented in Table 3 and fig 2.

The level of total serum Cholesterol(mg%) of C_1 group in collected blood samples at 0 , 5^{th} , 10^{th} , 15^{th} and 21^{st} day were 142.41 ± 1.56, 143.40 ± 1.24, 145.10 ± 1.46, 145.13 ± 1.21,146.10 ± 1.31 respectively. The level of total serum Cholesterol in C_2 group were 167.78 ±1.27,168.42±1.46,169.35±1.18, 169.95±1.20,170.25 ± 1.25 at 0,5^{th} ,10^{th} ,15^{th} , 21^{st} day of blood serum collection respectively. The level of total serum Cholesterol in T_1 group were143.32± 2.38, 145.43±2.34, 151.05± 1.27, 165.85±4.08, 168.85± 2.23 and in T_2 group were 143.28±4.05,144.85±4.05, 150.32± 1.37, 158.23±1.48,159.75±2.48 at 0day ,5^{th} day ,10^{th}day ,15^{th} day ,21^{st} day of blood serum collection respectively.

Analysis of variance (Table - 4) revealed significant variation between treatment groups but non-significant effect among periods of blood collection.

On zero days, the mean values of total serum Cholesterol (mg %) in serum samples were 142.41± 1.56 and 167.78±1.27 in C_1&C_2 respectively. The mean total serum Cholesterol of C_1, T_1, & T_2 groups showed significantly ($P \leq 0.01$) lowered value by 25.37, 24.46,&24.50 from the normal cyclic control group (C_2). However, the mean total serum Cholesterol value did not differ significantly between anoestrous control group (C_1) and both treated group T_1 &T_2 . The level of total serum Cholesterol in zero day collection did not show significant variation between both treatments groups T_1 & T_2.

On 5th day collection, the mean value of total serum Cholesterol (mg %) was found to be 143.40±1.24 and 168.42± 1.46 in C_1&C_2 respectively. Then mean total serum Cholesterol value of C_1 , T_1 & T_2 group showed significantly($P \leq 0.01$) lowered value by 25.02,22.99 &23.57 respectively from the normal cyclic control group(C_2) whereas, the mean total serum Cholesterol of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 2.03 and 1.45 from anoestrous control group (C_1).The level of total serum Cholesterol in 5^{th} day collection did not show significant variation between both treatments groups T_1 & T_2.

The trend of 10th day collection was similar to that of 5th day collection. The mean value of total serum Cholesterol (mg %) in serum samples were 145.10±1.46 and 169.35±1.18 in C_1&C_2 respectively. The mean total serum Cholesterol value of C_1 , T_1 &T_2 group showed significantly($P \leq 0.01$) lowered value by 24.25, 18.30 and 19.03 from the normal cyclic control group (C_2). The mean total serum Cholesterol value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 5.95 & 5.22 from anoestrous control group (C_1) whereas, the level of total serum Cholesterol in 10th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 15th day of collection, the mean value of total serum Cholesterol (mg %) in serum samples was found to be 145.13±1.21 and 169.95±1.20 in C_1&C_2 respectively. The mean total serum Cholesterol of C_1 ,T_1 & T_2 showed significantly ($P \leq 0.01$) lowered value by 24.82, 4.10 and 11.72 respectively from the normal cyclic control group (C_2). The mean total serum Cholesterol value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 20.72 & 13.10 from anoestrous control group (C_1)whereas, the level of total serum Cholesterol in 15th day collection showed significant variation between both treatments groups T_1 & T_2.

The trend of 21st day collection was similar to that of 15th day collection. The mean value of total serum Cholesterol (mg %) in serum samples were 146.10±1.31 and 170.25±1.25 in C_1&C_2 respectively. The mean value of total serum Cholesterol in C_1, T_1 & T_2 group showed significantly ($P \leq 0.01$) lowered value by 24.15,1.40 and10.50 from the normal cyclic control group (C_2). However, the mean total serum Cholesterol value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 22.75 & 13.65 from anoestrous control group (C_1). The level of total serum Cholesterol in 21st day collection showed significant variation between both treatments groups T_1 & T_2.

Serum calcium:

The mean ± S.E. of serum calcium in various groups have been presented in Table 5 & fig 3.

The level of serum calcium (mg%) in collected blood samples of C_1 group at 0 day, 5th day , 10th day , 15th day and 21st day of blood collection, were 9.65 ± 0.11, 9.65± 0.11, 9.67±0.11, 9.68± 0.11, 9.75±0.11 respectively. The level of serum calcium in C_2 group were 10.05± 0.51,10.08±0.50, 10.15±0.48, 10.17± 0.49, 10.18±0.46 at 0 day,5th day,10th day,15th day, 21st day of blood serum collection respectively. The level of serum calcium in T_1 groups were 9.69± 0.26,10.03±0.25,10.75±0.25,10.78±0.26,10.83±0.26 and in T_2 group were 9.58±0.21,10.02±0.20,10.62±0.25,10.74±0.10,10.88±0.21 at 0 day ,5th day, 10th day ,15th day and 21st day of blood serum collection respectively.

Analysis of variance (Table - 6) revealed significant variation between treatment groups but non-significant effect among periods of blood collection.

On zero day, the mean values of serum calcium (mg %) in serum samples were found to be 9.65±0.11 and 10.05±0.51 in C_1&C_2 respectively. The mean serum calcium of C_1, T_1, & T_2 groups showed significantly ($P\leq 0.01$) lowered values by 0.40, 0.36, and 0.47 from the normal cyclic control group (C_2). However, the mean serum calcium value did not differ significantly between anoestrous control group (C_1) and both treated groups T_1 &T_2 . The level of serum calcium in zero day collection did not show significant variation between both treatments groups T_1 & T_2.

On 5th day collection, the mean value of serum calcium (mg %) in serum samples were 9.65±0.11 and 10.08±0.50 in C_1&C_2 respectively. The mean serum calcium value of C_1, T_1& T_2 groups showed significantly ($P\leq 0.01$) lowered value by 0.43, 0.05, and 0.06 from the normal cyclic control group (C_2) However, the mean serum calcium value of T_1 and T_2 groups showed significantly($P\leq 0.01$)higher value by 0.38 and 0.37 from anoestrous control group (C_1).The level of serum

calcium in 5th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 10th day collection, the mean value of serum calcium (mg %) in serum samples were found 9.67±0.11 and 10.15±0.48 in C_1&C_2 respectively. The mean serum calcium value of C_1 showed significantly (P≤ 0.01) lowered value by 0.48 from the normal cyclic control group (C_2) whereas, T_1 & T_2 group showed significantly (P≤ 0.01) higher value by 0.60 and 0.47 from the normal cyclic control group (C_2). The mean serum calcium value of T_1 and T_2 group showed significantly(P≤0.01) higher value by 1.08 & 0.95 from anoestrous control group (C_1). The level of serum calcium in 10th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 15th day of collection, the mean value of serum calcium (mg %) in serum samples were found to be 9.68±0.11 and 10.17±0.49 in C_1&C_2 respectively. The mean serum calcium value of C_1 showed significantly(P≤ 0.01) lowered value by 0.49 from the normal cyclic control group (C_2) whereas, T_1 &T_2 showed significantly higher value by 0.61 and0.57 from the normal cyclic control group (C_2). The mean serum calcium value of T_1 and T_2 group showed significantly(P≤0.01) higher value by1.10 & 1.06 from anoestrous control group (C_1).The level of serum calcium in 15th day collection did not show significant variation between both treatments groups T_1 & T_2.

The level of serum calcium at 21st day collection was almost similar to that of 15th day collection. The mean value of serum calcium (mg %) in serum samples were 9.75±0.11 and 10.18±0.46 in C_1&C_2 respectively. The mean serum calcium value of C_1 showed significantly (P≤ 0.01) lowered value by 0.43 from the normal cyclic control group (C_2) but T_1 & T_2 groups showed significantly(P≤0.01) higher value by 0.65 and 0.70. The mean serum calcium value of T_1 and T_2 group showed significantly(P≤0.01) higher value by 1.08 & 1.13 from anoestrous control group (C_1). The level of serum calcium in 21st day

collection did not show significant variation between both treatments groups T_1 & T_2.

Serum Inorganic Phosphorus:

The mean ± S.E. of serum Phosphorus in various groups have been presented in Table 7 and fig 4.

The level of serum Phosphorus(mg%) of C_1 group in blood samples collected 0 day,5th day , 10th day ,15th day and 21st day were 3.12± 0.02, 3.14±0.03, 3.17±0.02, 3.19±0.02, 3.22±0.02 respectively. The level of serum Phosphorus(mg%) in C_2 group were 3.48 ± 0.01,3.49 ± 0.01,3.51 ± 0.01,3.52 ± 0.01,3.54 ± 0.01 at 0 day,5th day,10th day,15th day and 21st day of blood serum collection respectively. The level of serum Phosphorus in T_1 groups were 3.15±0.02,3.17±0.03,3.33±0.04,3.50±0.01,3.57±0.01and in T_2 groups were 3.17±0.03,3.20±0.03,3.33 ±0.04,3.41±0.30,3.46±0.02 at 0 day,5th ,10th 15th and 21st day of blood serum collection respectively.

Analysis of variance (Table -8) revealed significant variation between both treatment groups and among periods of blood collection.

On zero day collection, the mean values of serum Phosphorus (mg %) in serum samples were found to vary from 3.12±0.02 and 3.48±0.01 in C_1&C_2 respectively. The mean value of serum Phosphorus in C_1, T_1 & T_2 groups showed significantly ($P \leq 0.01$) lowered value by 0.36, 0.33, and 0.31 from the normal cyclic control group (C_2). However, the mean serum Phosphorus value did not differ significantly between anoestrous control group (C_1) and treated group T_1 &T_2 groups. The level of serum Phosphorus did not show significant variation between both treatments groups T_1 & T_2.

On 5th day collection, the mean value of serum Phosphorus (mg %) in serum samples were recorded as 3.14±0.03 and 3.49±0.01 in C_1&C_2 respectively. The mean serum Phosphorus in C_1, T_1 & T_2 group showed significantly ($P \leq 0.01$) lowered value by 0.35, 0.32 and 0.29 respectively from the normal cyclic

control group (C_2). The mean value of serum Phosphorus in T_2 group showed significantly($P \leq 0.01$) higher value by 0.06 from anoestrous control group (C_1) whereas, its level in T_1group did not show significant variation from anoestrous control group (C_1).The level of serum Phosphorus showed significant variation between both treatments groups T_1 & T_2.

On 10th day collection, the mean value of serum Phosphorus (mg %) in serum samples were found to be from 3.17±0.02 and 3.51±0.01 in C_1&C_2 respectively. The mean serum Phosphorus in C_1 ,T_1 & T_2 group showed significantly ($P \leq 0.01$) lowered value by 0.34, 0.18, and 0.18 respectively from the normal cyclic control group (C_2) . The mean serum Phosphorus value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.16 & 0.16 from anoestrous control group (C_1) comparison within treatment groups showed that the level of serum Phosphorus in 10th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 15th day of collection, the mean value of serum Phosphorus (mg %) in serum samples were found from 3.19±0.02 and 3.52±0.01 in C_1&C_2 respectively. The mean serum Phosphorus of C_1 and T_2 groups, showed significantly ($P \leq 0.01$) lowered value by 0.33 and 0.11 from the normal cyclic control group (C_2) whereas, the level of serum phosphorus in T_1 group did not show significant variation from the normal cyclic control group (C_2) . The mean serum Phosphorus of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.31 & 0.22 from anoestrous control group (C_1).Comparision within that the level of serum Phosphorus varied significantly between both treatments groups T_1 & T_2.

On 21st day collection, the mean value of serum Phosphorus (mg %) in serum samples were 3.22±0.02 and 3.54±0.01 in C_1&C_2 respectively. The mean serum Phosphorus of C_1, & T_2 showed significantly($P \leq 0.01$) lowered value by 0.32 and 0.08 from the normal cyclic control group (C_2) but its level in T_1

group showed significantly(P≤0.01) higher value by 0.03 from the normal cyclic control group (C_2).The mean serum Phosphorus value of T_1 and T_2 group showed significantly(P≤0.01) higher value by 0.35 and 0.24 from anoestrous control group (C_1) whereas, the level of serum Phosphorus in 21st day collection showed significant variation within these treatments groups T_1 & T_2.

Serum Magnesium:

The mean ± S.E. of serum magnesium in various groups have been presented in Table 9 and fig 5.

The level of serum magnesium(mg%) in collected blood samples of C_1 group at 0 day, 5th day, 10th day ,15th day and 21st day of blood serum collection were 2.00 ± 0.12, 2.00±0.12, 2.01±0.12, 2.02±0.12, 2.01±0.12 respectively. The level of serum magnesium(mg%) in C_2 group were 2.39 ± 0.26,2.39 ± 0.26,2.38 ± 0.22,2.39 ± 0.22,2.40 ± 0.22 at 0 day,5th day,10th day,15th day, 21st day of blood serum samples respectively.

The level of serum magnesium(mg%) in T_1 groups were 2.05 ± 0.20 2.29±0.26,2.61±0.19,2.71 ± 0.20,2.76±0.21 and in T_2 group were 2.04 ±0.20,2.24± 0.26,2.67± 0.13,2.73± 0.13,2.75± 0.13 at 0day,5th day 10th day ,15th day ,21st day of collected blood serum samples respectively.

Analysis of variance (Table -10) revealed significant variation between treatment but non-significant effect among periods of blood collection.

On zero day collection, the mean values of serum magnesium (mg %) in serum samples were recorded as 2.00±0.12 and 2.39±0.26 in C_1&C_2 group respectively. The mean serum magnesium of C_1, T_1, & T_2 groups showed significantly(P≤ 0.01) lowered value by 0.39, 0.34, and 0.35 from the normal cyclic control group (C_2). However, the mean serum magnesium value did not differ significantly between anoestrous control group (C_1) and treated group T_1 &T_2 groups. The level of serum magnesium in zero day collection did not show significant variation between both treatments groups T_1 & T_2.

On 5th day collection, the mean value of serum magnesium (mg %) in serum samples were found to be 2.00±0.12 and 2.39±0.26 in C_1&C_2 group respectively. The mean serum magnesium value of C_1, T_1& T_2 groups showed significantly (P≤ 0.01) lowered value by 0.39, 0.10, and 0.05 from the normal cyclic control group (C_2). However, the mean serum magnesium value of T_1 and T_2 group showed significantly(P≤0.01) higher value by 0.29 and 0.24 from anoestrous control group (C_1). The level of serum magnesium in 5th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 10th day collection, the mean value of serum magnesium (mg %) in serum samples were 2.01±0.12 and 2.38±0.22 in C_1&C_2 respectively. The mean value of serum magnesium of C_1 group showed significantly(P≤ 0.01) lowered value (P ≤ 0.01) by0.37 from the normal cyclic control group (C_2) but T_1 & T_2 group showed significantly(P≤0.01) higher value (P ≤ 0.01) by0.23 and 0.29 from the normal cyclic control group (C_2). The mean serum magnesium value of T_1 and T_2 group showed significantly(P≤ 0.01) higher value by 0.60 & 0.66 from anoestrous control group (C_1). The level of serum magnesium in 10th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 15th day of collection, the mean value of serum magnesium (mg %) in serum samples were found 2.02±0.12 and 2.39±0.22 in C_1&C_2 group respectively. The mean value of serum magnesium of C_1 group showed significantly(P≤ 0.01) lowered value by 0.37 from the normal cyclic control group (C_2) whereas, T_1&T_2 did not show significant variation from the normal cyclic control group (C_2). The mean serum magnesium value of T_1 and T_2 group showed significantly(P≤ 0.01) higher value by 0.69 & 0.71 from anoestrous control group (C_1).The level of serum magnesium in 15th day collection did not show significant variation between both treatments groups T_1 & T_2. On 21st day collection, the mean value of serum magnesium (mg %) in

serum samples were recorded as 2.01±0.12 and 2.40±0.22 in C_1&C_2 group respectively.

The mean serum magnesium value of C_1 showed significantly ($P \leq 0.01$) lowered value by 0.39 from the normal cyclic control group (C_2) whereas, T_1 & T_2 groups showed significantly($P \leq 0.01$) higher value by 0.36 and 0.35. However, the mean serum magnesium value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.75 & 0.74 from anoestrous control group (C_1).The level of serum magnesium in 21st day collected samples did not show significant variation between both treatments groups T_1 & T_2.

Serum Zinc:

The mean ± S.E. of serum zinc in various groups have been presented in Table 11 and fig 6.

The level of serum zinc (PPM) in collected blood serum of C_1 group at 0 day , 5th day,10th day ,15th day and 21st day of blood serum collection were 0.89± 0.01, 0.89±0.01, 0.87±0.01, 0.86±0.01, 0.89±0.01 respectively. The level of serum zinc (PPM) in C_2 group were 1.73 ± 0.04,1.74 ± 0.03,1.77 ± 0.03,1.78 ± 0.02,1.80 ± 0.02 at 0 day,5th day,10th day,15th day, 21st day of blood serum collection respectively .The level of serum zinc (PPM) in T_1 groups were 0.89±0.08,1.45±0.08,1.58±0.08,1.62±0.08,1.63±0.08 whereas, the level of serum zinc in T_2 groups 0.87±0.08,1.38±0.08,1.58 ±0.08,1.63±0.08,1.66±0.07 at 0 day,5th ,10th ,15th ,21st day of blood serum collection respectively.

Analysis of variance (Table -12) revealed significant variation between treatment but non-significant effect among the periods of blood collection.

On zero day collection, the mean values of serum zinc (PPM) in serum samples were found to be from 0.89±0.01 and 1.73±0.04 in C_1&C_2 group respectively. The mean serum zinc of C_1, T_1, & T_2 groups showed significantly ($P \leq 0.01$) lowered value by 0.84, 0.84, and 0.87 from the normal cyclic control group (C_2). The mean serum zinc value did not differ significantly between anoestrous control group (C_1) and treated group T_1 &T_2 groups. The level of serum zinc in zero day

collection did not show significant variation between both treatments groups T_1 & T_2.

On 5th day collection, the mean value of serum zinc (PPM) in serum samples were recorded as 0.89±0.01 and 1.74±0.03 in C_1&C_2 group respectively. The mean serum zinc value of C_1, T_1 & T_2 group showed significantly ($P \leq 0.01$) lowered value by 0.85, 0.29, and 0.36 from the normal cyclic control group (C_2). The mean serum zinc value in T_2 group showed significant variation from anoestrous control group (C_1) but the mean value of zinc in T_1 did not show significant variation from anoestrous control group (C_1).The level of serum zinc in 5th day collected samples showed significant variation between both treatments groups T_1 & T_2.

On 10th day collection, the mean value of serum zinc (PPM) in serum samples were observed as 0.87±0.01 and 1.77±0.03 in C_1&C_2 group respectively. The mean serum zinc value of C_1 ,T_1 & T_2, showed significantly($P \leq 0.01$) lowered value by 0.90, 0.19, 0.19 from the normal cyclic control group (C_2) .The mean serum zinc value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.71 & 0.71 from anoestrous control group (C_1). The level of serum zinc in 10th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 15th day of collection, the mean value of serum zinc (PPM) in serum samples were found 0.86±0.01 and 1.78±0.02 in C_1&C_2 group respectively. The mean serum zinc value of C_1, T_1 & T_2 showed significantly ($P \leq 0.01$) lowered value by 0.92, 0.16, and 0.15 from the normal cyclic control group (C_2). The mean serum zinc value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.76 & 0.77 from anoestrous control group (C_1).The level of serum zinc did not show significant variation within both treatments groups T_1 & T_2.

On 21st day collection, the mean value of serum zinc (PPM) in serum samples were recorded as 0.89±0.01 and 1.80±0.02 in C_1&C_2 group respectively. The mean serum zinc value of C_1, T_1 & T_2 showed significantly ($P \leq 0.01$) lowered value by 0.91, 0.17, and0.14 from the normal cyclic control group (C_2). The mean serum zinc value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.74 & 0.77 from anoestrous control group (C_1).The level of serum zinc did not show significant variation between both treatments groups T_1 & T_2.

Serum Manganese:

The mean ± S.E. of serum Manganese in various groups have been presented in Table (13) and fig(7).

The level of serum Manganese(PPM) in C_1 group in blood serum samples collected at 0 day, 5th day , 10th day , 15th day and 21st day were 0.94± 0.03, 0.94±0.03, 0.95±0.03, 0.96±0.03, 0.95±0.03 respectively. The level of serum Manganese(PPM) in C_2 group were 1.38 ± 0.13,1.40 ± 0.13,1.43 ± 0.12,1.46 ± 0.13,1.49 ± 0.12 at 0 day,5th day,10th day,15th day, 21st day of blood serum collection respectively.The level of serum Manganese(PPM) in T_1 groups were 0.96±0.13,1.22±0.11,1.54±0.11,1.55±0.11,1.56±0.11and in T_2 groups 0.92±0.10,1.20±0.09,1.52 ±0.01,1.53±0.01,1.53±0.09 at 0 day,5th ,10th ,15th 21st day of blood serum collection respectively.

Analysis of variance (Table -14) revealed significant variation between treatment groups but non-significant effect among periods of blood collection.

The mean values of serum Manganese (PPM) in serum samples collected at zero day were found to be 0.94±0.03 and 1.38±0.13 in C_1&C_2 respectively. The mean serum Manganese of C_1, T_1& T_2 groups showed significantly ($P \leq 0.01$) lowered value by 0.44, 0.42, and 0.46 from the normal cyclic control group (C_2). However, the mean value of serum Manganese did not differ significantly between anoestrous control group (C_1) and treated group T_1 &T_2 . The level of serum Manganese in samples of zero day collection did not show significant variation between both treatments groups T_1 & T_2.

On 5th day collection, the mean value of serum Manganese (PPM) in serum samples were noted as 0.94±0.03 and 1.40±0.13 in C_1&C_2 respectively. The mean serum Manganese value of C_1, T_1 & T_2 group showed significantly ($P \leq 0.01$) lowered value by 0.46, 0.18and 0.20 from the normal cyclic control group(C_2) . However, the mean serum Manganese value of T_1& T_2 group showed significantly($P \leq 0.01$) higher value by 0.28and0.26 from anoestrous control group (C_1). The level of serum Manganese in blood samples of 5th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 10th day collection, the mean value of serum Manganese (PPM) in serum samples were recorded as 0.95±0.03 and 1.43±0.12 in C_1&C_2 group respectively. The mean serum Manganese value of C_1 showed significantly ($P \leq 0.01$) lowered value by0.48 from the normal cyclic control group (C_2) whereas,T_1 & T_2 group did not show significant variation from the normal cyclic control group (C_2). The mean serum Manganese value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.59 & 0.57 from anoestrous control group (C_1). The level of serum Manganese in 10th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 15th day of collection, the mean value of serum Manganese (PPM) in serum samples were 0.96±0.03 and1.46±0.13 in C_1&C_2 group respectively. The mean value of serum Manganese in C_1 group showed significantly($P \leq 0.01$) lowered value by 0.50 from the normal cyclic control group (C_2) but T_1 and T_2 group did not show significant variation from the normal cyclic control group (C_2).The mean value of serum Manganese of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.59 & 0.57 from anoestrous control group (C_1). The level of serum Manganese in 15th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 21st day collection, the mean value of serum Manganese (PPM) in serum samples were found to be 0.95±0.03 and 1.49±0.12 in C_1&C_2 group respectively. The mean value of serum Manganese in of C_1 group showed significantly($P \leq 0.01$) lowered value by 0.54 from normal cyclic control group (C_2) whereas, T_1 and T_2 group did not show significant variation from the normal cyclic control group (C_2).The mean value of serum Manganese of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.61 & 0.58 from anoestrous control group (C_1).The level of serum Manganese in blood samples of 21st day collection did not show significant variation between both treatments groups T_1 & T_2.

Serum copper:

The mean ± S.E. of serum copper in various groups have been presented in Table 15 and fig 8.

The level of serum copper(PPM) in collected blood samples of C_1 group at 0 day,5th day, 10th day ,15th day and 21st day of blood serum collection were 1.30± 0.04, 1.31±0.04, 1.32±0.04, 1.33±0.09, 1.35±0.04 respectively. The level of serum copper in C_2 group were 1.53 ± 0.01,1.55 ± 0.01,1.56 ± 0.01,1.57 ± 0.01,1.58 ± 0.00at 0 day,5th day,10th day,15th day, 21st day of blood serum collection respectively. The level of serum copper in T_1 groups were1.31±0.04,1.61±0.08,1.67±0.77,1.69±0.07,1.70±0.07andinT_2groups1.29±0.03,1.62±0.03,1.63 ±0.03,1.65±0.04,1.68±0.04 at 0 day,5th ,10th ,15th ,21st day after blood serum collection respectively.

Analysis of variance (Table -16) revealed significant variation between treatment group but non-significant effect among periods of blood collection.

On zero day collection, the mean values of serum copper (PPM) in serum samples were found 1.30±0.04 and 1.53±0.01 in C_1&C_2 group respectively. The mean serum copper of C_1, T_1& T_2 groups showed significantly ($P \leq 0.01$) lowered values by 0.23, 0.22, and 0.24 from the normal cyclic control group (C_2). However, the mean serum copper value did not differ significantly between anoestrous control group (C_1) and treated group T_1 &T_2 groups. The level of

serum copper in blood samples collected at zero day did not show significant variation between both treatments groups T_1 & T_2.

On 5th day collection, the mean value of serum copper (PPM) in serum samples were recorded as 1.31±0.04 and 1.55±0.01 in C_1&C_2 group respectively. The mean serum copper value of C_1 groups showed significantly($P \leq 0.01$) lowered value by 0.24 from the normal cyclic control group (C_2) but the level of copper in group T_1 & T_2 did not show significant variation from the normal cyclic control group (C_2). The mean serum copper value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.30 and 0.31 from anoestrous control group (C_1).The level of serum copper in 5th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 10th day collection, the mean value of serum copper (PPM) in serum samples were found 1.32±0.04 and 1.56±0.01 in C_1&C_2 group respectively. The mean serum copper value of C_1 showed significantly($P \leq 0.01$) lowered value by0.24 from the normal cyclic control group (C_2) whereas, the level of serum copper in T_1 & T_2 group showed significantly($P \leq 0.01$) higher value by 0.11 and 0.07 from the normal cyclic control group (C_2). The mean serum copper value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.35 & 0.31 from anoestrous control group (C_1). The level of serum copper in 10th day collection did not show significant variation between both treatments groups T_1 & T_2.

The mean value of serum copper (PPM) in serum samples on 15th day of collection, were 1.33±0.09 and 1.57±0.01 in C_1&C_2 group respectively. The mean serum copper value of C_1 group showed significantly($P \leq 0.01$) lowered value by 0.24 from the normal cyclic control group (C_2) whereas,T_1&T_2 showed significantly higher value by 0.12 and 0.08 from the normal cyclic control group (C_2). The mean serum copper value of T_1 and T_2 group showed significantly higher value by0.36 & 0.32 from anoestrous control group

(C_1).The level of serum copper in 15th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 21st day collection, the mean value of serum copper (PPM) in serum samples were found 1.35±0.04 and 1.58±0.00 in C_1&C_2 group respectively. The mean serum copper value in C_1 group showed significantly($P \leq 0.01$) lowered value by 0.23 from the normal cyclic control group (C_2) but T_1 & T_2 groups showed significantly($P \leq 0.01$) higher value by 0.12 and 0.10. However, the mean serum copper value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.35 & 0.33 from anoestrous control group (C_1).The level of serum copper in 21st day collection did not show significant variation between both treatments groups T_1 & T_2.

Serum Cobalt:

The mean ± S.E. of serum Cobalt in various groups have been presented Table 17 and fig 9.

The level of serum Cobalt (PPM) in C_1 group in blood serum samples collected at 0 day, 5th day, 10th day ,15th day and 21st day of collection were 0.52± 0.02, 0.53±0.02, 0.53±0.02, 0.53±0.02, 0.54±0.01 respectively. The level of serum Cobalt in C_2 group were 0.61 ± 0.01,0.62 ± 0.01,0.62 ± 0.01,0.63 ± 0.01,0.63 ± 0.01 at 0 day,5th day,10th day,15th day, 21st day after blood serum collection respectively. The level of serum Cobalt in T_1 groups were 0.51±0.01,0.64±0.03,0.75±0.03,0.86±0.03,0.88±0.03and in T_2 groups were 0.53±0.01,0.68±0.04,0.79 ±0.04,0.81±0.04,0.83±0.04 at 0 day,5th ,10th ,15th 21st day after blood serum collection respectively.

Analysis of variance (Table -18) revealed significant variation between treatment groups but non-significant effect among periods of blood collection.

On zero day collection, the mean values of serum Cobalt (PPM) in serum samples were found 0.52±0.02 and 0.61±0.01 in C_1&C_2 group respectively. The mean serum Cobalt of C_1, T_1, & T_2 groups showed significantly ($P \leq 0.01$) lowered value by 0.09, 0.10, and 0.08 from the normal cyclic control group

(C_2) However, the mean value of serum Cobalt did not differ significantly between anoestrous control group (C_1) and treated group T_1 &T_2 groups. The level of serum Cobalt in zero day collection did not show significant variation between both treatments groups T_1 & T_2.

On 5th day collection, the mean values of serum Cobalt (PPM) in serum samples were 0.53±0.02 and 0.62±0.01 in C_1&C_2 group respectively. The mean serum Cobalt value of C_1 group showed significantly (P≤ 0.01) lowered value by 0.09 from the normal cyclic control group (C_2) but T_1 & T_2 did not show significant variation from the normal cyclic control group (C_2). However, the mean serum Cobalt value of T_1& T_2 group showed significantly(P≤ 0.01) higher value by 0.11and0.15 from anoestrous control group (C_1).The level of serum Cobalt in 5th day collection did not show significant variation between both treatments groups T_1 & T_2.

On 10th day collection, the mean value of serum Cobalt (PPM) in serum samples were observed as 0.53±0.02 and 0.62±0.01 in C_1&C_2 group respectively. The mean serum Cobalt in C_1 group showed significantly (P≤ 0.01) lowered value by 0.09 from the normal cyclic control group (C_2) whereas, its level in T_1 & T_2 group showed significantly(P≤ 0.01) higher value by 0.13 and 0.17 respectively from the normal cyclic control group (C_2) . The mean serum Cobalt of T_1 and T_2 group showed significantly(P≤ 0.01) higher value by 0.22 & 0.26 in comparison of anoestrous control group (C_1). However, the level of serum Cobalt in these treatment groups showed significant variation between both treatments groups T_1 & T_2.

The mean value of serum Cobalt (PPM) in serum samples on 15th day of collection were found to be 0.53±0.02 and 0.63±0.01 in C_1&C_2 group respectively. The mean serum Cobalt value of C_1, showed significantly (P≤ 0.01) lowered value by 0.10 from the normal cyclic control group (C_2) whereas, it value in T_1 and T_2 group showed significantly(P≤ 0.01) higher value by 0.23

and 0.18 from the normal cyclic control group (C_2) . The mean serum Cobalt value of T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.33 & 0.28 from anoestrous control group (C_1). However, the level of serum Cobalt did not show significant variation between both treatments groups T_1 & T_2.

On 21st day collection, the mean value of serum Cobalt (PPM) in serum samples were recorded as 0.54±0.01 and 0.63±0.01in C_1&C_2 group respectively. The mean value serum Cobalt in C_1 group showed significantly($P \leq 0.01$) lowered value by 0.09 from normal cyclic control group (C_2) whereas, its level in T_1 and T_2 group showed significantly($P \leq 0.01$) higher value by 0.25 and 0.20 from the normal cyclic control group (C_2). The mean serum Cobalt value of T_2 group showed significantly($P \leq 0.01$) higher value by 0.29 but T_1 did not show significant variation from anoestrous control group (C_1).Comparison within treatment groups showed that the level of serum Cobalt varied significantly between both treatments groups T_1 & T_2.

Induction of oestrus & conception following different therapeutic measure in crossbred cattle.

All 30 anoestrus cross bred cows selected and kept in 3 groups viz. T_1, T_2 and C_1 were utilized to access the effect of administration of hormonal and non-hormonal drugs on induction of oestrus and subsequently the percentage of conception. Normal saline was injected in C_1 group of anoestrus crossbred cows.

The effect of those utilized drugs on induction of oestrus as well as conception rate was presented in table 19. The percentage of oestrus and conception in Human placental extract treated group and clomiphene-citrate treated group were 90%;88.88% and 70%;66.66% respectively. In comparison of 0% oestrus and conception in non-treated control group.

Effect on Induction of oestrus :

The efficacy of both utilized hormonal and non-hormonal drugs on induction of oestrus in cross bred cow was presented in table 20.The effect of different drugs on induction of oestrus was found to be significant($P \leq 0.05$).The induction of oestrus in Human placental extract treated anoestrus cross bred cows(T_1) was found to be 90% it was significantly($P \leq 0.05$) higher than the anoestrus cross bred cows treated with clomiphene –citrate (T_2) by 20 percent.

Effect on conception rate:

The efficacy of different drugs on conception rate in anoestrus cross bred cow following both hormonal and non-hormonal treatment was presented in Table 21. The effect of those utilized drugs on conception rate was found to be significant ($P \leq 0.05$).The conception rate of anoestrus cross bred cows treated with Human placental extract(T_1) was found to be 88.88% which was significantly ($P \leq 0.05$) higher than the anoestrus cross bred cows treated with clomiphene-citrate (T_2) by 17.46%.

5 DISCUSSION

Main objectives of the present studies were to observe the effect of administration of hormonal and non-hormonal products on changes in total protein, total cholesterol, calcium, inorganic phosphorus, magnesium, zinc, manganese, copper and cobalt in blood serum collected on 0 day, 5th day, 10th day, 15th day& 21st day after following of the treatment. Besides that, the effect of both hormonal and non-hormonal products were accessed on induction of oestrus and conception rate in anoestrus crossbred cows. The experiment was carried by maintaining four groups of animals in which two groups i.e. Human placental extract group (T_1), Clomiphene-Citrate group (T_2) were kept as treatment groups and two controls groups. Anoestrus control group (C_1) was utilized to access the comparative efficacy of both hormonal and non-hormonal drugs from treatment groups on induction of oestrus as well as accessing the comparative variation in different biochemicals profile. Besides that, the variation in biochemicals profile viz. serum total protein, total cholesterol, serum calcium, serum inorganic phosphorus, serum magnesium, serum zinc, serum manganese, serum copper, serum cobalt between anoestrus and normal cycling cows were accessed by comparing their variation in between C_1 and C_2.

Blood Biochemical Profiles:

Serum total protein:

The mean value of serum total protein in placentrex treated group (T_1) showed an increasing trend and were found to be 6.77±0.07 and 7.38±0.10gm% in blood serum samples collected at 0 day and 21st day of collection. The level

of serum total protein in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 6.84±0.09 and 7.48±0.94gm% at 0 day and 21st day respectively. The corresponding values of serum total protein in untreated anoestrus cows (C_1) were 6.87±0.24 and 6.90±0.24gm% at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in both T_1 and T_2 groups showed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups showed the non-significant variation within themselves though, significant increase in the level of serum total protein were recorded at different days of collection from 0 to 21st day in both treatment groups.

The present findings are in close agreement with finding of Purohit *et. al.*, (1993) who recorded an increasing trend in the level of serum total protein following Clomiphene-citrate in anoestrus cows. The finding of Singh *et. al.*, (2006) also showed almost similar variation in the level of serum total protein in pre- treated anoestrus cows (6.81±0.15) in comparison of induced estrus cows with GnRH (8.1gm%).

Protein deficiency may delay puberty and hamper post-partum reproductive performance due to reduced gonadotropin release and decrease anterior pituitary responsiveness to GnRH. Deficiency of protein also suppresses the synthesis and secretion of LH but not the secretary activities of FSH cells. Protein deficiency as well as its excessive intake causes acyclia and low conception rates.Patil and Deshpande (1979) reported that optimal protein level is necessary for the development of body sex organ and for expression of oestrus signs. Low level of serum protein affects the process of implantation (Roberts, 1971).Accordingly an increase in level of serum total protein after

administration of both hormonal and non-hormonal drugs in the present findings might had the similar influences of increased gonadotropin.

The mean value of total protein in anoestrous crossbred cow (C_1) was found to be varied from 6.87±0.24 and 6.90±0.24 (gm %) in collected blood serum samples at 0 and 21st day of collection but its variations were found non-significant. The corresponding mean value of serum protein in normal cyclic crossbred cows (C_2) were also showed non-significant variation from 7.46±0.08gm% at 0day of collection to 7.49±0.08gm% at 21st day of collection. Comparison in the level of total serum protein between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant($P \leq 0.01$) variations, which were in accordance to the findings reported by Sharma *et. al.*, (1984), Kumar *et. al.*, (1987), Naidu and Rao (1982), Venkatamunnichetty and Rao (1986), Baruah *et. al.*, (1988).However, Pareek and Aminuddin (1985), Agarwal *et. al.*, (1985) did not found any significant difference in the level of total serum protein in between anoestrus and normal cycling Rathi cows and this might be due to variations in breed of the animals as well as environmental conditions in which the experiment was conducted. The low level of total serum protein in anoestrus conditions as observed in the present findings might had caused deficiency of certain amino acids required for synthesis of gonadotropin as reported by Vohra *et. al.*, (1995) which lead to cessation of follicular activity.

The level of total serum protein at different days of collections in anoestrus crossbred cows and normal cyclic cows were incomparable because of unavailability of similar references on this aspect. The mean value recorded at different days of collection in the present studies in both C_1&C_2 groups were almost in accordance to the mean values of total serum protein recorded by Baruah *et. al.*, (1988), Venkatamunnichetty and Rao (1987). Agarwal *et. al.*, (1985) however, observed the higher level of total serum protein in anoestrus

(8.43±1.22gm %) and normal cyclic crossbred cows (8.86±1.52gm %) whereas, Kavani *et. al.,* (1987) recorded the lower level of total serum protein (5.65±0.25gm %) in anoestrus Kankrej heifer in comparison of (7.14±0.22gm %) in normal cyclic heifers. Such type of small variation in the level of total serum proteins observed by different scientist might be due to variation in breed and age of experimental animals besides seasonal variations in which the experiments were conducted.

Total Serum Cholesterol:

The mean value of cholesterol in placentrex treated group (T_1) showed an increasing trend and were found 143.32±2.38mg% and 168.85±2.23mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum cholesterol in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 143.28±4.05 mg% and 159.75±2.48 mg% at 0 and 21st day of collection. The corresponding values of total serum cholesterol in untreated anoestrus cows (C_1) ranged from 142.41±1.56mg% at 0 day of collection to 146.10±1.31mg% at 21st day of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group respectively showed significant ($P \leq 0.01$) variation in both the treatment groups. However, collectionwise comparison between these treatment groups showed the non-significant variation within themselves at 0,5th and 10th day of collection but showed significant variation at 15th and 21st day of collection though increase in the level of total serum cholesterol was recorded in both of the treatment group from 0 to 21st days of collection. The poor level of serum cholesterol in anoestrous animal might have been due to effect of pituitary and gonadal hormones as postulated by Robinson (1957). Accordingly an increase in level of total cholesterol after administration of both hormonal

and non-hormonal drugs in the present findings might had the similar influences of increased gonadotropin.

However, Khasatiya *et. al.,* (2005) recorded significantly lower level of total cholesterol level in GnRH treated group than control group. The level of total cholesterol in treated group at different duration of blood collection after clomiphene –citrate treatment were incomparable because of unavailability of references, though increased level of serum cholesterol after following of treatment might had shown the positive responses as cholesterol being an important precursor for the synthesis of steroid hormones.Nayak *et. al*., (2006) had also observed the significant increase ($P \leq 0.01$) in the level of serum cholesterol indicating stimulation of endocrine mechanism for restoration of ovarian activity.

Mean value of serum cholesterol in anoestrous crossbred cow (C_1) though was found to be varied 142.41±1.56 and 146.10± 1.31(mg %) in collected blood serum samples at 0 and 21st day of collection but these variations were found non-significant. The corresponding mean value of serum cholesterol in normal cyclic crossbred cows (C_2) also showed non-significant variation from 167.78±1.27 at 0 day of collection to170.25±1.25 at 21st day of collection. Comparison in the level of total cholesterol between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant($P \leq 0.01$) variations, which were in accordance to the findings reported by Aminudeen *et.al*.,(1984),Reddy (1984), Sharma *et. al*.,(1984),Kavani *et. al*.,(1987),Kumar *et. al*., (1987), Nair *et. al.*, (1987) and Pal *et. al*., (1991).

The level of total cholesterol at different days of collections in anoestrus crossbred cows and normal cyclic cows were incomparable because of unavailability of similar references on this aspect. The mean value recorded at different days of collection in the present studies in both C_1&C_2 groups were

almost in accordance to the mean values of total serum cholesterol recorded by Aminudeen *et. al.*, (1984).Reddy(1984) observed the higher level of total cholesterol in anoestrus cows (180.23mg/100ml) and normal cycling crossbred cows (236.1mg/100ml) whereas, Nair *et. al.*,(1987) recorded the lower level of total cholesterol of 73.33 mg% in anoestrus crossbred cows in comparison of 116.39mg% in normal cycling cows.

Serum calcium:

The mean value of serum calcium in placentrex treated group (T_1) showed an increasing trend and were 9.69±0.26 and 10.83±0.26mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum calcium in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 9.58±0.21and10.88±0.21mg% at 0 and 21st day of blood collection. The corresponding values of serum calcium in untreated anoestrus cows (C_1) ranged from 9.65±0.11mg% at 0 day to 9.75±0.11mg% 21st day of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group showed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison in the level of calcium between these treatment groups showed non-significant variation within themselves though, significant increase in the level of serum calcium were recorded at different days of collection from 0 to 21st day in both treatment groups. The present findings corroborated with findings of Dantre *et. al.*, (1998) and Singh *et. al.*, (2006) who also reported significant increase in calcium levels following GnRH treatment. Purohit *et. al.*, (1993) recorded significantly higher serum calcium level of clomiphene-citrate treated cows (11.30±0.825) mg% than anoestrus cows (10.47±0.904) mg%.The increase level of calcium recorded in the present finding following both hormonal and non-hormonal therapy had probably

involved in the calcium dependent mechanism and influencing the release of gonadotropins. Hurley and done (1989) reported that GnRH responsible for stimulation of LH release from pituitary cells involves calcium dependent mechanism of LH release and not released in absence of calcium or in presence of calcium blocking agents.

Mean value of serum calcium in anoestrous crossbred cow (C_1) was found to be varied from 9.65±0.11 and 9.75± 0.11(mg %) in collected blood serum samples at 0 and 21st day of collection. However, variation in the level of serum calcium at different days of collection were found non-significant. The corresponding mean value of serum calcium in normal cycling crossbred cows (C_2) also showed non-significant variation from10.05±0.51 at 0 day of collection to 10.18±0.46 at 21st day of collection. Comparison in the level of serum calcium between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant($P \leq 0.01$) variations, which were in accordance to the finding reported by Sharma *et.al*.,(1984), Prasad *et. al*.,(1984)and Kumar *et. al*.,(1986).However, Dabas *et. al*.,(1987),Nayak and Mohanty (1985)and Samad *et. al*.,(1980) did not found any significant difference in the level of serum calcium in between anoestrus and normal cyclic cows and this might be due to variations in breed of the animals as well as individual variation.

Serum Inorganic phosphorus:

The mean value of serum inorganic phosphorus in placentrex treated group (T_1) showed an increasing trend and were 3.15±0.03mg% and 3.57±0.01mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum inorganic phosphorus in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 3.17±0.03mg% and 3.46±0.02mg% at 0 and 21st day respectively. The level of

serum inorganic phosphorus in untreated anoestrus cows (C_1) showed little variation and ranged from 3.12±0.02mg% at 0 day of collection to 3.22±0.02mg% at 21st day of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group showed significant ($P \leq 0.01$) variation in both the treatment groups and among different periods of blood collection. However, comparison in efficacy of those hormonal and non-hormonal drugs between these treatment groups showed the non-significant variation within themselves at 0 day and 10th day of collection whereas, significant variation in the level of inorganic phosphorus was recorded at 5th ,15th ,21st day of collection. The present findings were in accordance with finding of Dantre *et. al.*, (1998) and Singh *et. al.*, (2006) who also reported significant increase in the level of inorganic phosphorus after administration of GnRH.Purohit *et. al.*, (1993) recorded significantly higher serum inorganic phosphorus in clomiphene-citrate treated group in comparison of anoestrus cows, which corroborate with the findings recorded in the present studies.

The level of phosphorus lead to delayed sexual maturity and cessation of oestrus cycle. Bhaskaran and Abdullah khan (1981) reported that even marginal deficiency of phosphorus may be detrimental to pituitary ovarian axis causing failure to onset of oestrus. Thus, the increase level of inorganic phosphorus after administration of both hormonal and non-hormonal drugs, especially after 10th day of administration of drugs, postulated the enhanced stimulation of pituitary ovarian axis leading to increase ovarian activity and ultimately onset of oestrus. Bhaskaran and Patil (1982) opinion that marginal deficiency of phosphorus can cause disturbance in pituitary ovarian axis including ovulation and irregular oestrus (Snook, 1958).

The mean value of serum inorganic phosphorus in anoestrous crossbred cow (C_1) was found to be varied from3.12±0.02 and 3.22± 0.02(mg %) in collected blood serum samples at 0 and 21st day of collection but its variations were found to be non-significant. The corresponding mean values of serum inorganic phosphorus in normal cyclic crossbred cows (C_2) also showed non-significant variation from3.48±0.01 at 0 day of collection to 3.54±0.01 at 21st day of collection. Comparison in the level of serum inorganic phosphorus between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant($P \leq 0.01$) variations, which were in accordance to the findings reported by Sharma *et. al.*, (1984), Reddy (1984) and Prasad *et. al.*, (1984). However, Agarwal *et. al.*,(1985),Nayak and Mohanty (1985)and Sivaih *et. al.*,(1986) did not found any significant difference in the level of serum inorganic phosphorus between anoestrus and normal cyclic cows and this might be due to variations in age and breed of the experimental animals.

The mean value recorded at different days of collection in the present studies in both C_1&C_2 groups are almost in accordance to the mean values of serum inorganic phosphorus recorded by Prasad *et. al.*, (1984).Reddy (1984) observed the higher level of serum inorganic phosphorus in both anoestrus (5.14mg/100ml) and normal cyclic crossbred cows (6.67mg/100ml)in comparison of the level of inorganic phosphorus, observed in the present findings. However, comparison in the level of inorganic phosphorus between anoestrus and normal cyclic group showed significant variation as recorded in the present findings. Such type of variations might be due to variation in physical conditions of experimental animals.

The level of serum inorganic phosphorus at different days of collections in anoestrus crossbred cows and normal cycling cows were incomparable because of unavailability of similar references on this aspect.

Serum Magnesium:

The mean value of serum Magnesium in placentrex treated group (T_1) showed an increasing trend and were 2.05±0.20 and 2.76±0.21mg% in blood serum samples collected at 0 day and 21st day of collections respectively. The level of serum Mg in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 2.04±0.20 and 2.75±0.13mg% at 0 and 21st day respectively. The corresponding values of serum Magnesium in untreated anoestrus cows (C_1) were 2.00±0.12 and 2.01±0.12mg% at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group respectively revealed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups showed the non-significant variation within themselves though, significant increase in the level of serum Magnesium were recorded at different duration of collection from 0 to 21st day. Though, after following of both placentrex treatment (GnRH) and clomiphene-citrate treatment in the present study. Khasatiya *et. al*., (2005) did not find the significant increase in level of serum Magnesium concentration after GnRH treatment, which might be due to individual variation in hormonal responsiveness by the utilized experimental animals. The significant variation in the level of serum Magnesium at oestrual stage (3.72±0.09) from non pregnancy stage (2.62±0.17) as reported by Bhattacharya *et. al.*, (1995) indicated an increase in Magnesium level after hormonal responsiveness and thus, indirectly substantiate the present finding. The level of serum Magnesium after clomiphene-citrate treatment was incomparable because of unavailability of reference.

The mean value of serum magnesium in anoestrous animal (C_1) was found to be varied from 2.00±0.12 at 0 day of collection to 2.01± 0.12(mg %) at

21st day in collected blood serum samples but its variations were found non-significant The corresponding mean value of serum magnesium in normal cyclic crossbred cows (C_2) were also showed non-significant variation from 2.39±0.26mg% at 0 day of collection to 2.40±0.22mg% at 21st day of collection. Comparison in the level of serum magnesium between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant($P \leq 0.01$) variations, which were in accordance to the findings reported by Kumar *et. al*.,(2009) who recorded significantly higher serum Magnesium in normal cyclic cows than anoestrus cows. However, Sanjay Raj *et. al*., (2006) did not find any significant variation in the level of serum Magnesium between anoestrus (1.685±0.005)mg/dl and cyclic Sahiwal heifers (1.681±0.010)mg/dl.

The level of serum magnesium (mg%) at 0 ,5th, 10th ,15th and 21st days of collections in anoestrus crossbred cows were 2.00±0.12, 2.00±0.12, 2.01±0.12, 2.02±0.12and 2.01±0.12 respectively. The level of serum Magnesium (mg%) in normal cyclic cows at 0,5th ,10th ,15th and 21st days of collection were 2.39±0.26,2.39±0.26,2.38±0.22,2.39±0.22and2.40±0.22 respectively which were incomparable because of unavailability of similar references on this aspect.

Serum Zinc:

The mean value of serum Zinc in placentrex treated group (T_1) showed an increasing trend and were found to be 0.89±0.08 and 1.63±0.08 (PPM) in blood serum samples collected at from 0 day and 21st day of collection. The level of serum Zinc in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.87±0.08 (PPM) and1.66±0.07(PPM) at 0 and 21st day respectively. The corresponding values of serum Zinc in untreated anoestrus cows (C_1) were 0.89±0.01(PPM) at 0 day of collection and 0.89±0.01 (PPM) at 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups showed significant ($P \leq 0.01$) variation in both the treatment groups. However, duration wise comparison within these treatment groups showed the non-significant variation within themselves at 0 day,10^{th} 15^{th}, 21^{st} day of collection but significant variation was recorded in blood samples collected at 5^{th} day of collection. The level of serum Zinc after treatment of hormonal and non-hormonal drug were incomparable because of unavailability of reference. However, higher level of serum zinc had reported to activates several enzyme systems and its deficiency lead to delayed puberty and lower conception rate (Kreplin,1992) and lower concentration of FSH and LH particularly in males (Boland,2003), which indirectly support the present findings.

The mean value of serum Zinc in anoestrous animal (C_1) was found to be 0.89±0.01 and 0.89± 0.01(PPM) in collected blood serum samples at 0 and 21^{st} day of collection. The corresponding mean value of serum Zinc in normal cyclic crossbred cows (C_2) were found1.73±0.04 at 0day of collection to1.80±0.02 at 21^{st} day of collection. Comparison in the level of serum Zinc between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P \leq 0.01$) variations. However, Singh *et. al.*, (2006) observed non- significant difference in the level of serum zinc between anoestrous and normal cyclic buffaloes whereas, Kumar *et. al.*, (2009) observed significantly ($P \leq 0.05$) higher zinc value in anoestrous cow than normal cyclic cow and this might be due to variations in species of the experimental animals.

Serum Manganese:

The mean value of serum Manganese in placentrex treated group (T_1) showed an increasing trend and were found to be 0.96±0.13 and 1.56±0.11 (PPM) in blood serum samples collected at 0 day and 21^{st} day of collection

respectively. The level of serum Manganese in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.92±0.10 at 0 day and 1.53±0.09(PPM) 21st day of collection. The corresponding values of serum Manganese in untreated anoestrus cows (C_1) were 0.94±0.03 and 0.95±0.03 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group revealed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups at various stages of collection showed non-significant variation within themselves though, significant increase in the level of serum Manganese were recorded at different days of collection from 0 to 21st day of collection in both treatment group. The level of serum Manganese after treatment of hormonal and non-hormonal drug were incomparable because of unavailability of reference. Function of Manganese in the synthesis of sterols and ultimately correlated with synthesis of gonadal hormones responsible for induction of oestrus had been reported by Nocek *et. al*., (2006) which indirectly favoured the higher level of Manganese at oestrual stage. Manganese is an important Co-factor for glycosyl transferase, Galactotranseferase & pyruvate carboxylase.Manganese function in synthesis of sterols can also be correlated with the synthesis of Gonadal steroid harmones.The pituitary are relatively rich in Mn and these organs are sensitive to its deficiency in diet (Hidiroglou, 1979).Increase uptake of Mn during 4-11 days of estrus cycle also suggests that it specifically involved in luteal metabolism. Reproductive performance may be altered by absent or irregular oestrous cycle decreased conception rate, higher abortion cases.

The mean value of serum Manganese in anoestrous crossbred cow (C_1) was found to be varied from 0.94±0.03 and 0.95± 0.03(PPM) in collected blood serum samples at 0 and 21st day of collection but its variations were found non-

significant. The corresponding mean value of serum Manganese in normal cycling crossbred cows (C_2) were also showed non-significant variation from 1.38±0.13(PPM) at 0 day of collection to 1.49±0.12(PPM) at 21st day of collection. Comparison in the level of serum Manganese between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant($P \leq 0.01$) variations. Kumar *et. al.*, (2009) observed that Manganese did not show any significant variation among the normal cyclic and anoestrous.

Serum Copper:

The mean value of serum Copper in placentrex treated group (T_1) showed an increasing trend and were found to be 1.31±0.04 and 1.70±0.07 (PPM) in blood serum samples collected at 0 day and 21st day of collection. The level of serum Copper in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 1.29±0.03and1.68±0.04 (PPM) at 0 and 21st day respectively. The corresponding values of serum Cu in untreated anoestrus cows (C_1) were 1.30±0.04 and 1.35±0.04 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups respectively showed significant ($P \leq 0.01$) variation in both the treatment groups.However, comparison between these treatment groups showed the non-significant variation at varies stages of collection within themselves though, significant increase in the level of serum Copper were recorded at different days of collection from 0 to 21st day in both treatment groups. The level of serum Cu after treatment of hormonal and non-hormonal drug were incomparable because unavailability of reference. Desai *et. al.*, (1978) had reported that the higher serum copper level had been associated with higher oestrogenic activity, leading to initiation of oestrus.Azad (2008) had also recorded higher level of serum copper in oestrus animals in comparison of

anoestrus animals. Bhattacharyya *et. al.,* (1995) also recorded significantly higher blood serum concentration of copper on the day of oestrus than other stages of reproduction. These observations indirectly favoured the findings, recorded in the present studies.

The mean value of serum Copper in anoestrous crossbred cow (C_1) was found to be varied from 1.30±0.04 and 1.35± 0.04(PPM) in collected blood serum samples from 0 and 21st day of collection but its variations were found non-significant. The corresponding mean value of serum Copper in normal cyclic crossbred cow (C_2) also showed non-significant variation from1.53±0.01(PPM) at 0 day of collection to 1.58±0.00(PPM) at 21st day of collection. Comparison in the level of serum Cu between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant($P\leq0.01$) variations which were almost similar to the findings reported by Singh *et. al.,* (2006) who observed significantly ($P\leq0.05$) lower serum level of Cu in anoestrus (0.59±0.03µg/ml) as compared to normal cyclic buffaloes. However, Kumar *et. al.,* (2009) observed that Cu did not show any significant variation between normal cyclic and anoestrous cows. Such type of variation might be due to variation in species of the utilized experimental animals. Copper deficiency leading to reproduction is hampered in a manner of depressed oestrous.Kreplin *et. al.*, (1992) reported that copper deficiency was associated with delayed puberty and thus, favouring the condition of anoestrus.

Serum Cobalt:

The mean value of serum Cobalt in placentrex treated group (T_1) showed an increasing trend and were found to be 0.51±0.01(PPM) and 0.88±0.03 (PPM) in blood serum samples collected at 0 day and 21st day of collection. The level of serum Cobalt in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.53±0.01(PPM) and0.83±0.04

(PPM) at 0 and 21st day respectively. The corresponding values of serum cobalt in untreated anoestrus cows (C_1) were 0.52±0.02 to 0.54±0.01 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups respectively showed significant ($P \leq 0.01$) variation in both the treatment groups. However, Comparison between these treatment groups at corresponding stages of collection showed the non-significant variation within themselves at 0, 5th and 15th day but significant variation was found at 10th and 21st day of collection. The influence of both hormonal and non-hormonal therapy on the level of serum cobalt at variation durations of collection were incomparable. However, the findings of (Hidiroglou, 1979) and Kreplin (1992) supported the present findings indirectly who reported reduction of oestrus due to deficiency of cobalt.

The mean value of serum Cobalt in anoestrous animal (C_1) was found to be varied and recorded as 0.52±0.02 and 0.54± 0.01(PPM) in blood serum samples at 0 and 21st day of collection but its variations were found non-significant. The corresponding mean value of serum Cobalt in normal cyclic crossbred cow (C_2) was found to be varied non-significantly and recorded as 0.61±0.01and 0.63±0.01(PPM) at 0 day and 21st day of collection respectively. Comparison in the level of serum cobalt between anoestrus crossbred cows (C_1) and normal cyclic crossbred cow (C_2) showed significant($P \leq 0.01$) variations ($P \leq 0.01$).However, Singh *et. al*., (2006) and Kumar *et. al*., (2009) found non-significant variation in the level of serum cobalt between anoestrous and normal cyclic cows.

Effect on induction of oestrus:

Human Placental Extract treated group (T_1) showed 90% induction of oestrus in anoestrus cross bred cows whereas, 70% induction of oestrus were recorded in clomiphene-citrate treated group (T_2).

Analysis of variance showing the effect of drugs showed significant ($P \leq 0.05$) variation in both the treatment group in comparison of non-treated control group(C_1).However, comparison in efficacy between those utilized drugs showed the significantly highered effect in T_1 group than T_2 group.

Results observed in the present study by following Human Placental Extract treatment(T_1) were, in closed agreement with finding of Prahalad *et. al.,* (2010) and Khasatiya *et. al.,*(2005) who recorded 80% and 83.33% induction of oestrus respectively in post-partum anoestrus buffalo with GnRH analogues treatment. However, Ahmed *et. al.,* (2003) and Usmani (2001) observed 40% and 27.8% exhibition of oestrus respectively in anoestrus cross bred cow with GnRH analogue treatment, which were lower than the present findings. The variation on induction of oestrus might be due to differences in responsiveness of hormonal drugs in utilized small number of treatment groups.

Result observed during the present study with clomiphene-citrate treatment was almost in accordance to the finding of Deshpande *et. al .,(*1976) Kodgali *et. al.,* (1980) ,Singh *et. al.,* (1984) and Purohit *et. al.,* (1993) who recorded 80%, 80.95%,100% and 83.3% induction of oestrus ,respectively by treating anoestrus cattle with clomiphene-citrate whereas, Kaikini *et. al.,* (1977) and Reddy *et. al.,* (1990) had recorded lower values of induction of oestrus (60%) in anoestrus cross bred cows after using clomiphene-citrate.

The variation observed in the present findings might be due to variation in species, breed and age as well as number of utilized experimental animals.

Effect on conception rate:

The percentage of conception in cross bred anoestrus cow in Human Placental Extract treated group (T_1) and clomiphene-citrate treated group (T_2) were recorded as 88.88% and 71.42% respectively.

Analysis of variance showing the effect of drugs on conception rate showed highly significant ($P \leq 0.05$) effect in comparison of non-treated control group.

Comparison on efficacy in terms of conception between utilized Human Placental Extract treated group (T_1) and clomiphene-citrate treated group (T_2) drugs showed 17.46% higher conception in (T_1) than T_2.

The observations recorded in the present study on effect of Human Placental Extract treatment on conception rate in anoestrus cross bred cow were in closed agreement with the finding of with Prahalad *et. al.*, (2010) who recorded 68.75% conception rate in anoestrus cross bred cows following the treatment with placentrex. However, Tamuli *et. al.*, (2002) and Ahmed *et. al.*, (2003) recorded higher conception rate of 87.5% and 75% respectively in anoestrus cross bred cow in placentrex treated group. The variation in conception rate recorded by those scientist might be due to variation in individual responsiveness of utilized drug in the utilized small number of experimental animals.

The observations recorded in the present study on effect of Clomiphene-citrate treatment on conception rate in anoestrus cross bred cow were nearly closed with approximate findings of Singh *et. al.*, (1984) and Kodgali *et. al.*, (1980) who recorded 100% and 60.31% conception rate in anoestrus cross bred cattle following the treatment with clomophene-citrate.However, Reddy *et. al.*, (1990) recorded lower conception rate of 55% in anoestrus cross bred cow in clomiphene-citrate treated group. The variation in conception rate recorded by

those scientist might be due to variation in individual responsiveness of utilized drug in the utilized small number of experimental animals.

Better results recorded with Placentrex during the present study might be attributed to the fact that it contained TsH-RH (Thyroid Stimulating Releasing Hormone) and CRF(Corticotropin Releasing Hormone) along with GnRH (Gonadotropin Releasing Hormone).TsH-RH which has been reported to stimulate the thyroid gland to release thyroxin which increases the basal metabolic rate and in turn stimulate the proper release of gonadotropic hormone (Roberts,1971 and McDonald,1980).The CRF has been reported to be synergistically active with the GnRH for the release of LH from the anterior pituitary (Baldwin and Sawyer 1974 and Caraty *et. al.*,1997) which helps in folliculogenesis.

2 REVIEW OF LITERATURE

I. PREVALENCE OF ANOESTRUS

Honnappa and Narayana (1986) found 2.6 per cent and 1.12percent smooth ovaries on examination of reproductive disorders of 25219 cattle during 1977-82 and 6103 cattle during 1981-84 respectively.

Kumar *et. al.*, 1986) studied on reproductive disorders of 810 non-descript cattle and the incidence of anoestrus was observed to be 19.25 per cent.

Nayak and Mohanty (1986)., obtained comparison to cows (24.87%) a higher incidence of true anoestrum in crossbred heifers (57.1%).

On examination of 331 cows Rahumathulla *et. al*., (1986) reported 5.4 percent of cows suffering from true anoestrum.

According to Singh and Singh (1986) 31.98 per cent proved true anoestrum out of 769 deshi cows on clinical examination while the highest incidence of anoestrus was recorded in winter (28.15%) as against the lowest in summer (21.23%).

Of the 1463 crossbred cattle (605 heifers, 858 cows) Chetty and Rao (1987) found 56.63 per cent infertility cases on examination out of which anoestrum condition was 13.05 per cent in heifers and 10.25 per cent in cows indicating higher incidence of anoestrus condition in heifers.

Hussain (1987) reported higher incidence of smooth ovaries in local cows (56.60%) as compared to crossbred ones (17.73%).

In a study on 546 Red Kandhari cows and their crossbreds Pargaonkar and Bakshi (1987) found 3 per cent and 2 per cent cases of anoestrus respectively.

Singh *et. al.,* (1987) reported the anoestrus among crossbred cattle (Holstein x Local of chotanagpur).The overall incidence of anoestrum was recorded to be 45.05 per cent. The incidence was higher (59.82%) in heifers than in cows (36.47%). They observed the highest number of anoestrus cases in May (67.50%) and the lowest in February (32.0%).

Mollah *et. al*., (1989) examined 1100 cows of breeding age (above 2 years) selected at random in several villages of Bangladesh. The study revealed 22.22 per cent (244) fertile and 77.77 per cent (856) infertile cows. Out of 856 infertile cattle, incidence of post-partum anoestrus was 57.24 per cent (490) in cows and 30.37 per cent (260) in heifers while the percentage of repeat breeder was recorded to be 12.39 per cent (106).

Sadat *et. al*., (1990) districts *Anantnag, Pulwama and Baramullah of Kashmir* of anoestrus to the extent of 36 per cent in a field examination of infertility cases of the found the incidence in which anoestrus due to malnutrition, non-specific cases and lactation effects were found to be 31.1 and 4 per cent respectively.

The incidence of anoestrous in Sahiwal, Rathi and Hariana cattle of Tarai region in Utter Pradesh were reported to be 42.30 per cent, 42.10 per cent and 63.33 per cent respectively by Saxena *et al.* (1991).

After examining 3427 crossbred cows of Kerala Iyer *et. al*., (1992) reported overall incidence of anoestrum to be 30.36 per cent.

Kumar and Kumar (1993) studied 310 cattle belonging to rural population of Tarai region of Uttar Pradesh, raised under traditional animal husbandry

practices of grazing and occasional concentrate feeding. They reported that 31.29 per cent of cows revealed true anoestrum.

II. BLOOD BIOCHEMICAL PROFILE:

A. TOTAL PROTEIN:

Naidu and Rao (1982) observed significantly lower level of protein in anoestrous heifers (7.17gm %) and 10 anoestrous cows (7.84gm %) than normal cycling heifers (9.2gm %).

Agarwal *et. al.*, (1982) reported lower serum total protein in repeater cows (7.55 ± 2.08; 6.19 ± 0.32 and 4.93 ± 0.34 g/dl) as compared to non-repeater (9.24 ± 0.71, 7.80 ± 0.51 and 5.24 ± 0.57 g/dl) crossbred cows on day 1, 13 and 16 of oestrus cycle respectively.

Sharma *et. al.*, (1984) reported an average (6.35±1.0gm %) of total protein in anoestrous crossbred cows which was significantly lower than the normal cycling animal (8.25±1.22gm %).

Agarwal *et. al.*, (1985) found no significant difference in the serum total protein level (8.43±1.22gm %) in anoestrus and (8.62±1.52gm %) in normal cycling crossbred cows.

Pareek and Aminuddin (1985) did not find any significant difference in serum protein content in anoestrus (7.6gm %) and normal cycling (7.99gm %) Rathi cows.

Venkatamunnichetty and Rao (1986) observed lower level of serum total protein content in both the anoestrous cows (7.06gm %) and heifers (7.16gm %) than normal cycling cows (8.22gm %) and heifers (8.18gm %).

Venkatamunnichetty and Rao (1987) estimated total protein in serum of normal prepubertal heifers and heifers with retarded body growth. They found that low level of protein in heifers with retarded growth (6.65±0.13gm %) than normal cycling heifers (7.41±0.12gm %).

Kavani, (1987) observed the total protein in anoestrus Kankrej was reported (5.65±0.25gm %) which was significantly lower than the normal cycling heifers (7.14±0.22gm %).

Kumar *et. al*., (1987) observed significantly lower total serum protein in anoestrus cows (6.48±0.20gm %) and in heifers (6.75±0.19gm %) as compared to normal cycling cows (7.17±0.08gm %) and heifers (6.96±0.18gm %), respectively.

Dutta *et. al*,. (1988) reported significantly lower level of serum total protein content in anoestrus cattle (6.58±0.42gm %) than normal cycling animals (8.76±0.30gm %).

Baruah *et.al*.,(1988) reported significantly lower serum protein in anoestrus Jersey heifers ranging from 6.23±0.26 to 6.92±0.16gm% than normal cycling heifers ranging from 7.19±0.42 to 8.08±0.42gm%.

Dutta *et. al*., (1991) evaluated the concentration of total serum protein (gm/dl) was towards lower side (9.17 ± 2.06) than oestrus group of cows.

Islam *et.al.*, (1994) observed significantly higher serum total protein concentration in normal crossbred cows on day 0 compared to 13 day of oestrus cycle.

Salem *et. al.*, (1994) reported non – significantly differences in blood protein concentration between normal and repeat breeder.

Burle *et. al.,* (1995) reported significantly higher serum total protein value in normally cycling than repeat breeding crossbred cows further they also reported a significant difference between (10.06 ± 0.17) and (8.57 ± 0.17) gm / dl and second day (7) 8.92 ± 0.18 and 7.97 ± 0.13 gm / dl blood sample in normal and repeat crossbred cows respectively.

Srivastava (1995) obtained a non significant difference in plasma total protein level in fertile (6.48 ± 0.09 gm/dl) and non fertile (6.68 ± 0.12 gm /dl) cows.

Ram Krishna (1996) recorded significantly lowered serum protein level in repeater (5.98 ± 0.09 gm/dl) compared to normally cycling (6.05 ± 0.18 g/dl) cows.

Tandle *et. al.,* (1997) reported that the serum total protein was significantly higher (7.74 ± 0.37 g %) in oestrus cows than anoestrus (4.41 ± 0.27 g %)

Singh and Pant (1998) studied blood biochemical profile in both normal and repeat breeder cows of rural farm and observed the level of total protein7.43 ± 0.15, 7.96 ± 0.15 and 7.60 ± 0.10 g% respectively.

Chandrahar *et. al.,* (2003) observed that the mean value of total serum protein was apparently lower in repeat breeder than normal cows, difference between two group was non significant.

Khasatiya *et. al.,* (2005) observed significantly higher blood plasma total protein after treatment with GnRH in true anoestrus buffalo.

Singh *et. al.*, (2006) found that value of total protein in anoestrus buffaloes and GnRH treated group were 6.81±0.15 and 8.1gm%, respectively.

B. CHOLESTEROL

Aminudeen *et. al.*, (1984) observed significantly lower volume of cholesterol in anoestrus cows (147.75±5.71mg/100ml) and in anoestrous heifers (177.50±3.79mg/100ml) over those in normal cycling cows (259.86±10.16mg/100ml) and normal cycling heifers (217.46±3.16mg/100ml) respectively.

Prasad *et. al.*, (1984) observed the cholesterol concentration to range from 107.95 to 321.89 mg% with an average of 224.44mg% in anoestrus crossbred cows. On the day of oestrus, the values were found to range from 163.68to327.95mg% with an average of 245.42mg%.

Reddy (1984) observed the level of cholesterol in normal cycling cows (236.1mg/100ml) were significantly higher as compared to anoestrous cows (180.23mg/100ml).

Sharma *et. al.*, (1984) observed lower level of serum cholesterol in anoestrus crossbred cows (91.15±6.28mg %) over those in normal cycling cows (99.62±8.23mg %).

Kavani *et. al.*, (1987) observed significantly lower cholesterol level in anoestrus kankrej heifers (169.54±6.08mg %) over the normal cycling ones (269.10±22.20mg %).

Kumar *et. al.*, (1987) non-descript anoestrus cows (69.82±2.62mg/100ml) and heifers (69.82±2.62mg/100ml) showed

significantly lower cholesterol level compared to normal cyclic cows (98.55±1.03mg/100ml)and heifers (99.26±1.46mg/100ml).

Awasthi *et. al.,* (1987) reported that average value of serum cholesterol level in normal cycling; fertile and infertile repeat breeder cows were 101.69 ± 7.55, 115.91 ± 8.28 and 118.84 ± 0.05 mg %, respectively, and statistically non-significant difference was recorded.

Nair *et. al.,* (1987) observed that the total serum cholesterol in the normal cycling cows was the highest (116.39 mg %). The anoestrus group had the lowest level of cholesterol (73.33 mg %) succeeded by the silent heat (92.44 mg %), nymphomanic (109.7 mg %) and finally repeat breeder (101.47 mg %) group of cows. A significant differences ($P<0.01$) was observed between the normal and abnormal cycling cows.

Pal *et. al.,* (1991) reported that the serum cholesterol exhibited a high significant ($P<0.01$) difference between cycling and non cycling heifers and cows.

Ramakrishna (1997) observed that the serum cholesterol level in anoestrus cows (80.94 ± 6.77) were significantly lower than cycling cows (108.94 ± 4.64).

Tandle *et. al.,* (1997) reported that the cholesterol concentration was significantly higher in oestrus cows (160 ± 6.78 mg %) than anoestrus cows (94.38 ± 6.59 mg %).

Chandrahar *et. al.,* (2003) observed that the serum cholesterol level was non significantly higher in repeat breeder cows (182.37 ± 6.29) than normal cycling cows (165.75 ± 11.1).

Shah *et. al.,* (2003) found that the level of total cholesterol did not differ significantly between postpartum fertile (180.25 ± 4.37 mg %) and infertile (188.25 ± 6.30 mg %) buffaloes.

Shah *et. al.,* (2003) found that the level of total cholesterol of GnRH treated buffalo were significantly higher (216.97 ± 9.88) in comparison to control untreated groups of 5-6 weeks (153.45 ± 11.75 mg %) and 13-14 weeks (263.48 ± 34.17) post partum buffaloes.

Khasatiya *et. al.,* (2005) observed that the level of blood total cholesterol after treatment with GnRH in true anoestrus buffalo were significantly lower (127.78 ± 4.33) than its control group (153.11 ± 5.91).

Patel *et. al.,* (2005) studied the blood biochemical profile following different hormonal therapies viz. GnRH, LH and progesterone in repeat breeding cows and recorded that weekly mean plasma total cholesterol levels in GnRH, LH and progesterone treated as well as control groups of repeat breeding cows varies significantly ($P<0.05$) in a narrow range with an overall mean of 221.29 ± 0.70 and 61.71 ± 0.63 mg/dl; respectively. They further recorded that the cholesterol level was significantly lowered ($P<0.05$) in GnRH treated group as compared to LH and progesterone treated and control groups. The mean cholesterol level in conceived and non-conceived groups of repeat breeding cows were 218.34 ± 1.15 and 223.79 ± 0.77 mg/dl ($P<0.01$).

Sharma *et. al.,* (2008) reported that the level of cholesterol (mg %) on 0, 7, 14, 21 days of oestrus cycle in the non-hormonally treated conceived cows were 119.63 ±16.26,115.10 ±

13.48,110.18±14.39 and 104.95± 12.03 respectively. Whereas the level of cholesterol (mg %) in hormonally treated conceived cows at corresponding days of collection were 88.70±4.70, 107.12±9.48, 103.32±11.70 and 95.22±7.12 respectively. Further, it was observed that the cholesterol level(mg%) in non conceived cow after following of the non-hormonal treatment were 106.10±5.83,106.62±6.2,98.02±4.49 and94.39±3.75 respectively at 0,7,14 and 21days of collection.Accordingly,the cholesterol level(mg%) in non conceived cow after following of the hormonal treatment were 99.13±6.65,105.00±8.09,101.96±6.07 and 94.70±7.22 respectively. Cholesterol concentration varied significantly between non-hormonally and hormonally treated repeat breeders as well as conceived and non conceived group of normal fertile buffaloes on all days of the cycle.

Khan *et. al.*, (2010) observed that the total serum cholesterol concentration in repeat breeding cows were significantly lower ($P<0.01$) when compared to normal cycling animals.

C. MACRO MINERALS:

(a) Calcium

Rao *et. al.*, (1981) observed no significant difference in serum Ca level in different stages of reproduction of normal cycling cow with mean value of (10.38 ± 0.35 mg/100ml).

Agarwal *et. al.*, (1982) observed the level of calcium (mg %) on day 1, 13 and 16 of oestrus cycle in repeat breeding cows 2.53 ± 0.17, 3.53 ± 0.18 and 4.94 ± 0.28 respectively. The corresponding

values in normal cycling cows were 3.20 ± 0.12, 3.60 ± 0.64 and 4.70 ± 0.99respectively.

Kulkarni *et al.* (1983) reported overall mean value of serum calcium (mg %) as 8.74 and 9.19 in Gir and crossbred lactating cows, respectively. The difference in the mean value of calcium in both the breeds was not found significant.

Naidu and Rao (1982) observed that the serum calcium level (10.00±1.65mg/100ml) in cows (10.14±1.72mg/100ml) as compared to anoestrous heifers (9.69±1.30mg/100ml) and cows (9.93±1.35mg/100ml).

Umashanker *et. al.*, (1983) reported that serum calcium concentration was higher in normal cyclic buffaloes (10.59 ± 2.59 mg/100ml) than repeat breeder buffaloes (8.44 ± 2.12 mg/100ml).

Prasad et al. (1984) reported significantly higher calcium value (10.18mg %) at oestrus as compared to anoestrus crossbred cows (9.97mg %).

Sharma *et. al.*, (1984) reported significant reduction in level of serum calcium in anoestrus crossbred cows (7.95±1.08mg %) as compared to normal cycling cows (10.69±2.05mg %).

Nayak and Mohanty (1985) found serum calcium level in anoestrus crossbred heifers and cows to be 9.42±0.27mg% respectively which was not significantly different in comparison to control.

Kumar *et. al.*, (1986) found significantly lower level of calcium in anoestrus cows (7.44±0.26mg %) and heifers (7.17±0.36mg %) as compared to normal cycling cows (8.98±0.22mg %) and heifers (9.20±0.34mg %), respectively.

Sivaih *et. al.*, (1986) estimated that there was no significant difference in level of calcium (8.15±2.46mg%) in oestrus and (7.48±2.14mg%) in anoestrus cows.

Kumar *et. al.*, (1986) recorded significantly lower serum calcium level in repeat breeder cows (7.05 ± 0.30 mg/100ml) in comparison to repeat breeder heifers (6.55 ± 0.40 mg/100ml) normal cyclic cows (8.98 ± 0.22 mg/100ml) and normal cyclic heifers (9.20 ± 0.34 mg/100ml).

Srivastava and Kharche (1986) reported lower calcium concentration during oestrus in normal cycling cows (10.50-15.00mg %) at corresponding stage of oestrus than abnormal cycling cows (repeater=9.00-16.00mg %)

Awasthi *et. al.*, (1987) reported the serum calcium level in normal cycling; fertile and infertile repeat breeder cows to be 7.86 ± 0.12, 8.93 ± 0.63 and 8.43 ± 0.77 mg %respectively, the difference among groups was non-significant.

Dabas *et. al.*, (1987) reported that serum concentrations of calcium didnot differ significantly between cycling crossbred cows (11.50±0.30mg/dl) and anoestrus crossbred cows (9.8±0.50mg/dl).

Singh and Vadnere (1987) found calcium in blood plasma to be significantly higher in oestrus crossbred than anoestrus crossbred cows.

Dutta *et. al.*, (1988) reported that serum calcium did not vary significantly between anoestrus Jersey heifers (10.73±0.06mg/100ml) and normal cycling Jersey ones (11.02±0.05mg/100ml).

Rupde *et. al.*, (1993) stated that serum calcium concentration in repeat breeder cows were 6.60 ± 2.56 and 7.32 ± 0.33mg% in pre-treatment and post treatment respectively. The mean values for regular breeding cow was 7.84 ± 0.65mg%.

Islam *et. al.*, (1994) found that the serum calcium levels were significantly lower in repeat breeders than in normal crossbred cows on 0 day and 13th day of oestrus.

Ramakrishna (1996) estimated serum calcium level in repeat breeding cows with uterine infection, repeat breeder cows without uterine infection and normal cycling cows and recorded the mean value of blood serum calcium as9.97 ± 0.52 mg%, 9.85 ± 0.21 mg%, and 9.95 ± 0.25 mg% respectively.

Singh *et. al.*, (1998) studied the blood biochemical profile of normal and repeat breeder cows in Himachal Pradesh and found that the values of calcium (mg%) were 8.42 ± 0.32 and 7.42 ± 0.31 in normal and repeat breeder cows respectively.

Kumar (2000) found that the serum calcium level did not differ significantly among various treated group viz.group supplemented with mineral mixture, Prostaglandin, Progesterone and GnRH administered group. Further it was observed that level did not differ at different stages of observation, i.e. day 0 (oestrus), 4, 14, 16 and 45hours of blood collection.

Das *et. al.*, (2002) recorded the serum calcium concentration in repeat breeder as 10.04 ± 0.32 and in normal cyclic cows as 10.50 ± 0.44 mg %.The difference in mean value of serum calcium did not differ significantly.

Shah *et. al.*, (2003) reported that the serum calcium level in GnRH treated group were significantly lower than control at 7-8 and 5-6 weeks postpartum.

Jyanthi *et. al.*, (2003) observed significantly lower calcium (10.03 ± 0.06 mg/dl) in repeat breeding as compared to normal control (10.95 ± 0.03) cows.

Chandrahar *et. al.*, (2003) found that serum calcium concentration was significantly higher in repeat breeder crossbred cows (9.63 ± 0.36) than in normal fertile crossbred cows (6.17 ± 0.17).

Singh *et. al.*, (2006) found that the values of serum calcium in anoestrus and GnRH treated buffaloes were 9.90±0.170 and 11.49±0.294mg/100ml,respectively.

(b) Inorganic Phosphorus:

Mokashi *et. al.*, (1974) studied serum phosphorus in post-partum anoestrus Gir cows. It was found to be significantly lower in anoestrus cows (5.24mg%) than in normal cycling ones (5.78mg%).

Rao *et. al.*, (1981) recorded mean serum inorganic phosphorus value in normal cows (6.11 ± 0.39 mg/100ml) which was slightly higher as compared to repeaters (6.82 ± 1.13 mg/100ml) but did not differ significantly.

Naidu and Rao (1982) estimated inorganic phosphorus level in normal cycling and anoestrus crossbred heifers and cows. Significant reduction in serum phosphorus level was found in anoestrus heifers (4.79±1.00mg/100ml) and cows

(4.65±1.08mg/100ml) as compared to normal cycling heifers (6.44±1.26mg/100ml) and cows (6.10±1.49mg/100ml)respectively.

Umashanker *et. al.*, (1983) observed higher (7.92 ± 1.18 mg/100ml) serum phosphorus concentration in normal cyclic as compared to repeat breeding (7.57 ± 1.12 mg/100ml) cows.

Aminudeen *et. al.*,(1984) in a study on normal cycling and anoestrus Rathi cattle of Rajasthan found significantly lower serum inorganic phosphorus in physically poor anoestrus cows (3.02±0.04mg/100ml) than in normal cycling ones (4.98±0.27mg/100ml). However, there was no significant difference between physically good anoestrus cows (5.01±0.21mg/100ml) and the normal cycling ones. Similarly there was no significant difference in inorganic phosphorus levels between anoestrus heifers (5.74±0.24mg/100ml and normal cycling ones (5.40±0.21mg/100ml).

Prasad *et. al.*, (1984) reported that in anoestrus crossbred cows inorganic phosphorus level in blood serum averaged 6.84mg% with range of 3.2 to 11.88mg% and in normal cycling cows it averaged 7.79mg% with range of 3.8 to 12.19mg%. The difference was found to be significant.

Reddy (1984) found a significantly higher concentration of inorganic phosphorus in normal cycling cows (6.67mg/100ml) compared to anoestrus cows (5.14mg/100ml) and heifers (5.24mg/100ml).

Sharma *et. al.*, (1984) reported a significant reduction in level of inorganic phosphorus in anoestrus crossbred cows

(2.97±0.23mg %) as compared to normal cycling crossbred ones (4.83±0.33mg %).

Nayak and Mohanty (1985) found a significantly lower level of inorganic phosphorus in anoestrus crossbred heifers (4.84±0.19mg %) and cows (4.87±0.27mg %) as compared to cycling heifers and cows. But the difference in value of anoestrus heifers and cows was found to be non-significant.

Kumar *et. al.,* (1986) reported level of serum phosphorus as 4.98 ± 0.06 mg/100ml in normal cyclic and 5.16 ± 0.21 mg/100ml. in repeater cows.

Mufarrege *et. al.,* (1986) reported that the blood phosphorus concentration was higher in cows which conceived than in those which did not conceive.

Srivastva *et. al.,* (1986) observed serum phosphorus concentration 6.60 mg% in repeater and 5.95% in normal cyclic buffaloes.

Awasthi *et. al.,* (1987) found that infertile repeaters had significantly lower (3.73 ± 0.29 mg %) inorganic phosphorus level than normal cyclic (5.06 ± 0.19 mg %) and fertile repeater (4.99 ± 0.25 mg %) cows.

Nair *et. al.,* (1984) stated that level of serum inorganic phosphorus in repeaters was 4.19 ± 0.14 mg percent significantly different from the normal cyclic (4.84 ± 0.2 mg percent) cows.

Khan *et. al.,* (1993) stated that regular breeding cows showed a higher (6.07 ± 0.15 mg %) level of serum inorganic phosphorus than repeat breeding (4.84 ± 0.1 mg %) cows.

Rupde *et. al.*, (1993) in his experiment on biochemical profile in repeat breeders found mean phosphorus concentration as 3.75 ± 0.22, 4.03 ± 0.21 in pre treatment and post treatment repeater respectively and for regular breeders cows were 4.46 ± 0.13mg%.

Islam *et. al.*, (1994) reported that the serum inorganic phosphorus levels were significantly lower in repeat breeders than in normal crossbred cows on 0 day and 13th day of oestrus.

Ramakrishna (1996) estimated mean serum inorganic phosphorus levels of 4.33 ± 0.14 mg% in repeat breeder cows without uterine infection and 5.96 ± 0.18 in normal cycling cows.

Singh and Pant (1998) reported that the level of phosphorus (mg %) was 6.27 ± 0.21 and 4.89 ± 0.14 in normal and repeat breeders cows respectively.

Kumar (2000) observed that the serum inorganic phosphorus level was significantly higher in normal cyclic cows than repeat breeding cows. The serum inorganic phosphorus level was also reported non-significantly higher in mineral mixture treated animal compared to untreated repeat breeder animals.

Das *et. al.*, (2002) found that the level of inorganic phosphorus was significantly lower in repeat breeders cow (4.72 ± 0.15 mg %) than normal cyclic cow (5.51 ± 0.26).

Chandrahar *et. al.*, (2003) studied on serum biochemical profile of repeat breeder and found that inorganic phosphorus in repeat breeder was (3.98 ± 0.05 mg %) lower than normal cycling cows (4.60 ± 0.04 mg %).

Shah *et. al.*, (2003) reported that the serum phosphorus level of GnRH treated group was significantly lower than control only at 7-8 and 5-6 weeks postpartum.

Jyanthi *et. al.*, (2003) found significantly lower phosphorus level in repeat breeding cows as compared to normal breeding cows. The level was 4.12 ± 0.03 mg/dl and 5.27 ± 0.04 mg/dl in repeat breeder and normal cycling cows respectively.

(c) Magnesium:

Dutta *et. al.*, (2001) observed the overall mean value for serum magnesium and found significantly ($P<0.01$) higher in cyclic heifers as compared to anoestrus heifers.

Khasatiya *et. al.*, (2005) observed that the serum magnesium concentration did not vary significantly between fertile (3.86 ± 0.08) and infertile (3.70 ± 0.09 mg/dl) buffaloes.

Khasatiya *et. al.*, (2005) recorded the level of blood plasma Magnesium 3.67±0.08 and 3.66±0.011mg/dl in true anoestrus and sub oestrus buffaloes respectively.

Kumar *et. al.*, (2009) reported that the serum magnesium was significantly ($P\leq0.05$) higher in normal cyclic cows than that in anoestrous cows.

Ahlawat *et. al.*, (2009) reported that the serum magnesium values did not differ significantly between normal cyclic (3.27 ± 0.18mg/dl) and non-cyclic (3.02 ± 0.00mg/dl) animals.

Chaurasia *et. al.*, (2010) reported that the concentration of total, free and bound form of magnesium did not vary significantly between repeat breeder and normal cyclic buffalo.

Tiwary *et. al.*, (2010) recorded average value of Magnesium in anoestrus cattle 2.04±0.91 mg/dl.

D. MICRO MINERALS

Parmar *et. al.*, (1986) found that plasma level of Manganese and Copper were significantly lower in repeat breeding cows than the normal cycling cows.

Das *et. al.*, (2002) observed that repeat breeder cows had significantly lower level of Cu (0.69 ± 0.017 µg / ml) and Zn (1.80 ± 0.033 µg / ml) than normal cyclic cows where the levels 0.97 ± 0.023 µg / ml and 2.09 ± 0.057 µg / ml. respectively. However, no significant variation was found in Mn between the groups.

Shah *et. al.*, (2003) reported that the Zinc level was significantly higher on the day of calving in GnRH treatment group than that in control group. Copper level was significantly higher only at 9-10 weeks postpartum in GnRH treated as compared to untreated group. However, week wise variation in the level of copper was found non significantly in both the groups. Manganese level in control group were significantly higher on the day of calving and within5 – 15 weeks, and the lowest on 3-4 weeks postpartum, the differences in level of Manganese among these groups were not significant.

Khasatiya *et. al.*, (2005) reported that the overall mean plasma copper and manganese levels in fertile and infertile groups of buffaloes were found to be (1.44 ± 0.03 Vs 1.44 ± 0.03 and 0.08

± 0.01 Vs 0.11 ± 0.01 PPM, respectively), which were the mean zinc level was significantly higher in further group (1.95 ± 0.06 Vs 1.66 ± 0.06 PPM) and cobalt lower (0.52 ± 0.02 Vs 0.61 ± 0.02 PPM) in infertile than the fertile group of buffaloes.

Singh *et. al.*, (2006) studied serum concentration of trace minerals was estimated in normal cyclic, anoestrus and repeat breeder buffaloes. The serum level of Zinc was lower in anoestrus(0.72±0.05µg/ml) and repeat breeder buffaloes (0.88±0.15µg/ml) as compared to normal cyclic buffaloes (1.00±0.04µg/ml).However, the difference was not significant. The serum level of Copper was significantly (P≤0.05) lower in anoestrus (0.59±0.03µg/ml) and repeat breeder buffaloes (0.62±0.03µg/ml) as compared to normal cyclic buffaloes (0.88±0.03µg/ml).The serum level of Cobalt was non-significantly lower in anoestrus (0.018±0.001µg/ml) and repeat breeder buffaloes (0.016±0.001µg/ml) as compared to normal cyclic buffaloes (0.022±0.002µg/ml).

Ceylan *et. al.*, (2008) found that the level of Zn and Cu in cows with repeat breeding and anoestrus were lower than in the normal cycling group, but the variations were not statistically significant.

Kumar *et. al.*, (2009) observed serum zinc value was significantly (P≤0.05) higher in anoestrus than normal cyclic cows and the serum levels of Copper, Cobalt and Manganese did not show any variation between normal cyclic and anoestrus cows.

III. NON-HARMONAL THERAPY

Deshpande *et. al.,* (1976) recorded onsets of induced heat within 4 to 11 days 80% of cows exhibited the symptoms of heat within 47 to 8 days of fertivet treatment with overall results at 53.65% especially during summer months.

Kulkarni *et. al.,* (1977) recorded treatment with Fertivet for inducing ovulation at proper time in 18 cows. The various dose levels tried were as follows (1)300mg/day for two day and 150mg on the 3rd day (2)450 mg/day for 2 day and 300mg on 3rd day and (3) 450mg/day for 3 day. The results of the treatment for induction of ovulation within normal limits as evidenced by the subsequent high percentage of conception, in groups second and third, with 75 and 71 respectively were very encouraging.

Kaikini *et. al.,* (1977) administered fertivet (300 mg/day) and manifestation of symptoms of heat were conspicuous in 60% of the cases. They further suggested that the above dose of fertivet therapy could give better result.

Dugwekar *et . al*., (1980) recorded five Brown swiss, 5 Jersey crossbred and Sahiwal cows which had been anoestrous for more than or equal to 3mth were each given 150ml of 1.0% Copper sulphate, and were then given 1 tablet of Fertivet (180mg cis-clomiphene citrate+ 120mg trans-clomiphene citrate) daily for 5 day or until they showed estrus.In the 3 groups 3,4 and 3 female resp. Showed estrus within 5 days of the end of treatment, and 5,4 and5 showed estrus within 15 days. The 60-day NR rate to insemination at estrus was 57.1%.

Singh *et .al.,* (1984) in a study on the drug trial for bringing anoestrus cattle in heat with fertivet, prajana, Lugol's solution, vetosterol and progesterone found fertivet to be the most effective. In cows 100% heat was observed within 8.82±2.90 days, where as only 72.7% heifers came in heat within 11.38±6.47 days with fertivet treatment. The pregnancy rate in cows and heifers were found to be 100% and 87.5% respectively. With prajana capsule

treated group 60% heat was observed within 31.17±6.80 days in cows, whereas 72.7% heifers came in heat within 21.38±5.81 days. The pregnancy rate in cows and heifers were found to be 66.67% and 87.50% respectively.

Reddy *et. al.,* (1990), studied the efficacy of "clofert-vet" in 20 anoestrus crossbred cows .After experiment recorded significantly($P \leq 0.01$) about60% of 20 anoestrus crossbred cows treated with clofert-vet exhibited ovulatory oestrus at an average interval of 8.42±0.98 days. While only 30% of 10 crossbred cows of control group evinced oestrus with a mean period of 30.33±3.39 days. They further recorded that overall conception rate in crossbred cows was 55.0% in treated group as against 30.0% in control group.

Purohit *et. al.,* (1993) studied fertivet treatment in Rathi cows and heifers. Fertivet was found to be more efficient in inducing fertile oestrous in true anoestrous Rathi cows (83.3%) than in past partum anoestrous Rathi cows (57.1%) and a shorter average span of 5.9 day was required in heifers as compared to average 8.75 days in cows for inducing oestrous. Analysis of blood glucose, serum calcium, serum phosphorous and total serum protein during fertivet induced oestrous showed higher values when compared with anoestrous but only serum calcium was significant statistically, however comparison of these values with those in normal oestrous cows showed no difference except for total serum protein which was significantly higher in normal oestrous cows.

EL-Sherry *et. al.,* (2011) studied the effect of Clomiphene-citrate on follicular development and super ovulation during the first follicular wave in Rahmani sheep with a 22-day ovulation cycle. They further recorded a significant ($P \leq 0.05$) increase in the number of follicle and in the levels of estradiol after clomiphene-citrate treatment.

IV. HORMONAL THERAPY:

Shams *et . al.,* (1991) observed that Receptal (5 ml, I/M) used in anestrous crossbred cows exhibited 100 percent estrus at an average interval of 21.00±6.37 days with 57.14 percent conception rate.

Reddy *et. al.,* (1994a) studied the use of gonadotrophic releasing hormone administered in this study as a single I/M injection and induced estrus in 50 percent cases with a mean interval of 10.80±3 days. However a low conception rate of 40 percent was recorded consequent to first A.I.

Thakur *et. al.,* (1996) recorded that out of ten buffaloes injected with receptal, 6 animals exhibited pronounced estrus on an average interval of 21.6 days with cent percent fertility. They concluded that Receptal even in reduced dose was able to induce estrus /Corpus luteum in treated animals with very good conception rate.

Dantre *et. al.,* (1998) reported that Receptal treated 85.71 percent heifers expressed oestrus within 23.5±1.89 days post treatment with 66.66 percent conception rate.

Tamuli and Tamuli (2001) reported the efficacy of patented human placental extract (HPE) in the treatment of infertile animals. All heifers 100 percent and 224 cows (97.4%) conceived with confirmation of pregnancy up to 97.6 percent required 1.3 number of services per conception.

Tamuli *et. al.,* (2002) used placentrex in HF C.B heifers, Jersey C.B heifers and Jersey C.B cow @ 10ml I/V for 3 days and in HF C.B cow @ 15ml I/V for 3 days.They recorded the average days required for induction of estrus and overall conception rate in Jersey C.B heifers, HF C.B cow and Jersey C.B cow were 14.2±5.2;73.33%, 18.5±1.6;75% and 11.7±4.3;87.5% respectively.

Ahmed *et. al.,* (2003) reported that total 10 anoestrus crossbred cows were treated with Placentrex at 15 ml, I/V daily for 3 days. They exhibited

oestrus within 8-13 days of post- treatment with an average interval of 10.25±0.85days.Three (75.00%) out of 4 induced cows conceived.

Shah *et. al.,* (2003) observed that the GnRH treated buffaloes resumed ovarian activity and expressed first postpartum oestrus significantly earlier than its control. Nearly 80% buffaloes ovulated within 15 days of GnRH treatment with 100% conception by 80 days postpartum and reduced calving interval indicating promising response towards improving reproductive efficiency.

Navanukraw *et. al.,* (2004) studied on presynchronization protocol and used GnRH and $PGF_2\alpha$ as schedule of ovisynch treatment and found 49.6% conception rate in cow.

Patel *et. al.,* (2005) observed the conception rate in repeat breeding in GnRH, LH/hCG and Progesterone treated group 66.66, 83.33 and 50.00% respectively.

Ahmed *et. al.,* (2007) concluded that intravenous use of placentrex was effective in repeat breeder with ovulatory disturbance. The overall success rate in this study was 88.88% in repeat breeders.

Bhattacharyya *et. al.,* (2009) reported that the conception rate in repeat breeding with clinical history of delayed ovulation was 66.67% in HCG treated group and 33.33% in Placentrex treated group.

Butani *et. al.,* (2009) studied on 53 repeat breeding and 12 normal cyclic buffaloes to evaluate the efficacy of hormonal and non-hormonal drugs in improving fertility and its effect on trace minerals profile. Among 14, 13 and 16 repeat breeding buffaloes treated with 0.02 mg GnRH I/M, just after Al; 500 mg of hydroxy-progesterone caproate I/M on day 4th or 5th post-Al, and antibiotics

i/m at Al (enrofloxacin) or I/U12-24 hrs post-Al (ceftriaxone), the conception rates in the treatment cycle were 57.14, 46.15 and 33.33%, respectively, against only 20% in untreated control repeat breeders (n=10) and 50% in normal cyclic group. The results of hormone therapy were better ($P<0.05$) and at par with the normal fertile group.

Prahlad *et. al.*, (2010) treated 80 buffaloes with various protocol (60 affected with postpartum anoestrum + 20 healthy cyclic buffaloes), the estrus response found to be 70, 90, 80 and 50 percent, respectively in GnRH, PMSG, Placentrex and Control group. The overall conception rate was better in PMSG (77.78 percent) group than GnRH (64.29 percent), Placentrex (68.75 percent) and control (40 percent) groups.

6 SUMMARY & CONCLUSION

The present study was carried out on 30 anoestrus crossbred cows and 10 normal cycling crossbred cows maintained at Composite livestock cattle farm, Bihar veterinary college, Patna; organised and un-organised private Khatals around Patna and cross-bred cows brought at veterinary clinical complex BVC Patna.

The anoestrus crossbred cows were selected on the basis of owner's history and repeated gynaeco-clinical examination of reproductive organs at 10 days intervals. Incorporation of normal cyclic crossbred cows was done on the basis of owner's history, examining colour and consistency of oestrual mucal discharge and per rectal examination of genital organs. All selected animals were dewormed with broad spectrum ecto parasitic drug and categorised into 4 groups.

Main objectives of the present studies were to observe the effect of administration of hormonal(Human placental extract) and non-hormonal(Clomiphene-citrate) products on changes in total protein, total cholesterol, calcium, inorganic phosphorus, magnesium, zinc, manganese, copper and cobalt in blood serum collected on 0 day, 5^{th} day, 10^{th} day, 15^{th} day& 21^{st} day after following of the treatment. Besides that, the effect of both hormonal and non-hormonal products were accessed on induction of oestrus and conception rate in anoestrus crossbred cows. The experiment was carried by maintaining four groups of animals in which two groups i.e. Human placenta extract treated group (T_1), Clomiphene-Citrate treated group (T_2) were kept as treatment groups and two controls groups C_1 and C_2 comprising of anoestrus

and normal cycling crossbred cows respectively. Anoestrus control group (C_1) was utilized to access the comparative efficacy of both hormonal and non-hormonal drugs on induction of oestrus as well as post-treatment comparative variation in the level of different biochemicals profile in comparison of treatment groups. Besides that, the normal cycling crossbred cows(C_2) were utilized for accessing variation in blood biochemicals profile viz. serum total protein, total cholesterol, serum calcium, serum inorganic phosphorus, serum magnesium, serum zinc, serum manganese, serum copper, serum cobalt in comparison of anoestrus crossbred cows on different durations of blood collection.

The mean value of serum total protein in placentrex treated group (T_1) showed an increasing trend and were found to be 6.77±0.07 and 7.38±0.10gm% in blood serum samples collected at 0 day and 21st day of collection respectively. The level of serum total protein in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 6.84±0.09 and 7.48±0.94gm% at 0 day and 21st day respectively. The corresponding values of serum total protein in untreated anoestrus cows (C_1) were 6.87±0.24 to 6.90±0.24gm% at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in both T_1 and T_2 groups showed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups showed the non-significant variation within themselves though, significant increase in the level of serum total protein were recorded at different days of collection from 0 to 21st day in both treatment groups.

The mean value of total protein in anoestrous crossbred cow (C_1) was found to be varied from 6.87±0.24 to 6.90±0.24 (gm %) in collected blood serum samples at 0 and 21st day of collection but its variations were found non-

significant. The corresponding mean value of serum protein in normal cyclic crossbred cows (C_2)were also showed non-significant variation from 7.46±0.08gm% at 0day of collection to 7.49±0.08gm% at 21st day of collection. Comparison in the level of total serum protein between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant ($P\leq 0.01$) variations.

The mean value of cholesterol in placentrex treated group (T_1) showed an increasing trend and were found 143.32±2.38mg% and 168.85±2.23mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum cholesterol in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 143.28±4.05 mg% and 159.75±2.48 mg% at 0 and 21st day of collection. The corresponding values of total serum cholesterol in untreated anoestrus cows (C_1) ranged from 142.41±1.56mg% at 0 day of collection to 146.10±1.31mg% at 21st days of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group respectively showed significant ($P\leq 0.01$) variation in both the treatment groups.

. Mean value of serum cholesterol in anoestrous crossbred cow (C_1) was found to be varied 142.41±1.56 and 146.10± 1.31(mg %) in collected blood serum samples at 0 and 21st day of collection but these variations were found non-significant. The mean value of serum cholesterol in normal cyclic crossbred cows (C_2) also showed non-significant variation from 167.78±1.27 at 0 day of collection to170.25±1.25 at 21st day of collection. Comparison in the level of total cholesterol between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant ($P\leq 0.01$) variations.

The mean value of serum calcium in placentrex treated group (T_1) showed an increasing trend and were 9.69±0.26 and 10.83±0.26mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum calcium in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 9.58±0.21and10.88±0.21mg% at 0 and 21st day of blood collection. The values of serum calcium in untreated anoestrus cows (C_1) ranged from 9.65±0.11mg% at 0 day to 9.75±0.11mg% 21st day of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group showed significant ($P\leq0.01$) variation in both the treatment groups. However, comparison in the level of calcium between these treatment groups showed non-significant variation within themselves though, significant increase in the level of serum calcium were recorded at different days of collection from 0 to 21st day in both treatment groups. Comparison in the level of serum calcium between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P\leq0.01$) variations.

The mean value of serum inorganic phosphorus in placentrex treated group (T_1) showed an increasing trend and were 3.15±0.03mg% and 3.57±0.01mg% in blood serum samples collected at 0 day and 21st day of collection. The level of serum inorganic phosphorus in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 3.17±0.03mg% and 3.46±0.02mg% at 0 and 21st day respectively. The level of serum inorganic phosphorus in untreated anoestrus cows (C_1) showed little variation and ranged from 3.12±0.02mg% at 0 day of collection to 3.22±0.02mg% at 21st day of collection.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group showed significant ($P\leq0.01$) variation in both the

treatment groups and among different periods of blood collection. However, comparison in efficacy of those hormonal and non-hormonal drugs between these treatment groups showed the non-significant variation within themselves at 0 day and 10^{th} day of collection whereas, significant($P \leq 0.01$) variation in the level of inorganic phosphorus was recorded at 5^{th} ,15^{th} ,21^{st} day of collection.

The mean value of serum inorganic phosphorus in anoestrous crossbred cow (C_1) was found to be varied from3.12±0.02 to 3.22± 0.02(mg %) in collected blood serum samples at 0 and 21^{st} day of collection but its variations were found to be non-significant. The mean values of serum inorganic phosphorus in normal cyclic crossbred cows (C_2) also showed non-significant variation from3.48±0.01 at 0 day of collection to 3.54±0.01 at 21^{st} day of collection. Comparison in the level of serum inorganic phosphorus between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P \leq 0.01$) variations.

The mean value of serum Magnesium in placentrex treated group (T_1) showed an increasing trend and were 2.05±0.20 and 2.76±0.21mg% in blood serum samples collected at 0 day and 21^{st} day of collections respectively. The level of serum Mg in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 2.04±0.20 and 2.75±0.13mg% at 0 and 21^{st} day respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group respectively revealed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups showed the non-significant variation within themselves though, significant increase in the level of serum Magnesium were recorded at different duration of collection from 0 to 21^{st} day. Comparison in the level of serum

magnesium between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P \leq 0.01$) variations.

The mean value of serum Zinc in placentrex treated group (T_1) showed an increasing trend and were found to be 0.89±0.08 and 1.63±0.08 (PPM) in blood serum samples collected at from 0 day and 21st day of collection. The level of serum Zinc in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.87±0.08 (PPM) and1.66±0.07 (PPM) at 0 and 21st day respectively. The corresponding values of serum Zinc in untreated anoestrus cows (C_1) were 0.89±0.01 (PPM) at 0 day of collection and 0.89±0.01 (PPM) at 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups showed significant ($P \leq 0.01$) variation in both the treatment groups. However, duration wise comparison within these treatment groups showed the non-significant variation within themselves at 0 day,10th 15th, 21st day of collection but significant($P \leq 0.01$) variation was recorded in blood samples collected at 5th day of collection. Comparison in the level of serum Zinc between anoestrus crossbred cows (C_1) and normal cyclic crossbred cows (C_2) showed significant ($P \leq 0.01$) variations.

The mean value of serum Manganese in placentrex treated group (T_1) showed an increasing trend and were found to be 0.96±0.13 and 1.56±0.11 (PPM) in blood serum samples collected at 0 day and 21st day of collection respectively. The level of serum Manganese in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.92±0.10 at 0 day and 1.53±0.09(PPM) 21st day of collection. The corresponding values of serum Manganese in untreated anoestrus cows (C_1) were 0.94±0.03 and 0.95±0.03 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 group revealed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups at various stages of collection showed non-significant variation within themselves though, significant increase in the level of serum Manganese were recorded at different days of collection from 0 to 21st day of collection in both treatment group.

The mean value of serum Manganese in anoestrous crossbred cow (C_1) was found to be varied from 0.94±0.03 and 0.95± 0.03(PPM) in collected blood serum samples at 0 and 21st day of collection but its variations were found non-significant. The corresponding mean value of serum Manganese in normal cycling crossbred cows (C_2) were also showed non-significant variation from 1.38±0.13 (PPM) at 0 day of collection to 1.49±0.12 (PPM) at 21st day of collection. Comparison in the level of serum Manganese between anoestrus crossbred cows (C_1) and normal cycling crossbred cows (C_2) showed significant ($P \leq 0.01$) variations.

The mean value of serum Copper in placentrex treated group (T_1) showed an increasing trend and were found to be 1.31±0.04 and 1.70±0.07 (PPM) in blood serum samples collected at 0 day and 21st day of collection. The level of serum Copper in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 1.29±0.03and1.68±0.04 (PPM) at 0 and 21st day respectively. The corresponding values of serum Cu in untreated anoestrus cows (C_1) were 1.30±0.04 and 1.35±0.04 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups showed significant ($P \leq 0.01$) variation in both the treatment groups. However, comparison between these treatment groups

showed the non-significant variation at different stages of collection within themselves though, significant increase in the level of serum Copper were recorded at different days of collection from 0 to 21st day in both treatment groups.

The mean value of serum Cobalt in placentrex treated group (T_1) showed an increasing trend and were found to be 0.51±0.01 (PPM) and 0.88±0.03 (PPM) in blood serum samples collected at 0 day and 21st day of collection. The level of serum Cobalt in Clomiphene-citrate treated group (T_2) also showed an increasing trend with corresponding value of 0.53±0.01 (PPM) and0.83±0.04 (PPM) at 0 and 21st day respectively. The corresponding values of serum cobalt in untreated anoestrus cows (C_1) were 0.52±0.02 to 0.54±0.01 (PPM) at 0 and 21st day of collection respectively.

Analysis of variance showing the effect of hormonal and non-hormonal therapy in T_1 and T_2 groups respectively showed significant ($P\leq0.01$) variation in both the treatment groups.

The mean value of serum Cobalt in anoestrous animal (C_1) was found to be varied and recorded as 0.52±0.02 and 0.54± 0.01(PPM) in blood serum samples at 0 and 21st day of collection but its variations were found non-significant. The corresponding mean value of serum Cobalt in normal cyclic crossbred cow (C_2) was found to be varied non-significantly and were recorded as 0.61±0.01and 0.63±0.01(PPM) at 0 day and 21st day of collection respectively. Comparison in the level of serum cobalt between anoestrus crossbred cows (C_1) and normal cyclic crossbred cow (C_2) showed significant ($P\leq0.01$) variations.

Based on findings, recorded in the present studies it could be concluded that the administration of both hormonal(Human placental extract) and non hormonal(Clomiphene-citrate) drugs caused significant($P\leq0.01$) increase in

post treated blood serum concentration of total protein, cholesterol, calcium, inorganic phosphorus, Magnesium, Zinc, Manganese,Copper and Cobalt. Which might had improved the hypophyseal gonadal functions and ultimately improved ovarian follicular activity resulting in initiation of oestrus. The increased level of total protein and different ionic concentrations viz. Inorganic phosphorus, Magnesium, Copper and Zinc, observed after administration of those two types of drugs were postulated to be involved in enhancing metabolic activity and creating favourable uterine environment besides stimulation of pituitary ovarian axis, resulting ovulation and increased percentage of conception.

Comparison on efficacy of induction of oestrus and the percentage of conception between those two utilized drugs showed 20.0% higher induction of oestrus and 17.46% increased conception rate after administration of placental extract in comparison of clomiphene-citrate treated group in which induction of oestrus and conception rate were found to be 90% and 88.88% respectively. Thus, it was concluded that administration of placental extract would be more effective for induction of oestrus and increasing of conception rate in anoestrus crossbred cows in agroclimatic condition of Bihar. However, further detailed investigation need to be conducted on larger population to explain the mechanism of varying biochemical profiles and mode of enhancing reproductive physiology by administration of those utilized hormonal and non-hormonal drugs.

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