Plate No 1: Clomiphene citrate with 1% CuSO₄ tablet.

Plate No 2: Phosphorus injection
Plate No 3: Vetade injection (Vitamin $\text{AD}_3\text{E}$)

Plate No 4: Receptal injection (GnRH Hormone)
THERAPEUTIC MANAGEMENT OF DELAYED PUBERTY IN CROSSBRED HEIFERS

THESIS

Submitted
in partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE
IN
ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BY

NAGARE SACHIN BHAGWAN
Enrolment. No. V/07/062

Bombay Veterinary College, Mumbai

MAHARASHTRA ANIMAL AND FISHERY SCIENCES
UNIVERSITY, NAGPUR - 440 001.
(INDIA)
2014
Appendix-B

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled “THERAPEUTIC MANAGEMENT OF DELAYED PUBERTY IN CROSSBRED HEIFERS” or part thereof has not been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publications of any university or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

Date: (NAGARE SACHIN BHAGWAN)
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Reg. No. 1448

Date: Dr. R. J. Chaudhari
Chairman
Advisory Committee
Assistant Professor
Animal Reproduction, Gynaecology
And Obstetrics.
Appendix - C (i)

DECLARATION OF ADVISORY COMMITTEE

Shri. NAGARE SACHIN BHAGWAN has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled, “THERAPEUTIC MANAGEMENT OF DELAYED PUBERTY IN CROSSBRED HEIFERS” submitted by him is the result of research work is sufficient to warrant its presentation to the examination in the subject of Department of ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS for the award of MASTER OF VETERINARY SCIENCE (M.V.SC) degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by him/her for a degree of any other University.

Place : Mumbai
Date :

Signature
(Dr. R. J. Chaudhari)
Chairman
Advisory Committee
Assistant Professor
Animal Reproduction, Gynaecology
And Obstetrics.

ADVISORY COMMITTEE

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CERTIFICATE

This is to certify that the thesis entitled, “THERAPEUTIC MANAGEMENT OF DELAYED PUBERTY IN CROSSBRED HEIFERS” submitted by Shri. NAGARE SACHIN BHAGWAN to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of MASTER OF VETERINARY SCIENCE (M.V.Sc.) has been approved by the Student’s Advisory Committee after examination in collaboration with the External Examiner.

Name and Signature of External Examiner Signature with Seal (Dr. R. J. Chaudhari) Head of Department Advisor/Guide

Advisory Committee

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Dean/Associate Dean
ACKNOWLEDGEMENTS

As I am writing my thesis in the last few days I went down the nostalgia lane and realized that acknowledgment is a perfect opportunity to thank the people without whom I would have been nothing today.

First of all, I am very thankful to my parents especially to my mother who supported and encourages me in every step of my education.

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I have no expression which would be able to be described in any language the thanks deserved by my friend Dr. Snehlata Baviskar who have been with me through thick and thin.

Place                                                                       Sachin Bhagwan Nagare

Date
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<tr>
<td>1</td>
<td>LH</td>
<td>Luteinizing Hormone</td>
</tr>
<tr>
<td>2</td>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>3</td>
<td>mm³</td>
<td>Cubic millimeter</td>
</tr>
<tr>
<td>4</td>
<td>Million/mm³</td>
<td>Million per cubic millimeter</td>
</tr>
<tr>
<td>5</td>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>6</td>
<td>gm/dL</td>
<td>Gram per deciliter</td>
</tr>
<tr>
<td>7</td>
<td>10⁶/L</td>
<td>Million per liter</td>
</tr>
<tr>
<td>8</td>
<td>PCV</td>
<td>Packed cell volume</td>
</tr>
<tr>
<td>9</td>
<td>MCV</td>
<td>Mean corpuscular volume</td>
</tr>
<tr>
<td>10</td>
<td>MCHC</td>
<td>Mean corpuscular hemoglobin concentration</td>
</tr>
<tr>
<td>11</td>
<td>TLC</td>
<td>Total Leukocyte Count</td>
</tr>
<tr>
<td>12</td>
<td>N</td>
<td>Neutrophil</td>
</tr>
<tr>
<td>13</td>
<td>L</td>
<td>Lymphocyte</td>
</tr>
<tr>
<td>14</td>
<td>E</td>
<td>Eosinophil</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>Monocyte</td>
</tr>
<tr>
<td>16</td>
<td>Lakhs/m³</td>
<td>Lakhs per cubic millimeter</td>
</tr>
<tr>
<td>17</td>
<td>fL</td>
<td>femtoliters</td>
</tr>
<tr>
<td>18</td>
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<tr>
<td>19</td>
<td>NS</td>
<td>Non significant</td>
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<tr>
<td>20</td>
<td>&gt;</td>
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<td>21</td>
<td>&lt;</td>
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<td>22</td>
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<td>23</td>
<td>i.e.</td>
<td>that is</td>
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<td>24</td>
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<td>Number</td>
</tr>
<tr>
<td>25</td>
<td>β</td>
<td>beta</td>
</tr>
<tr>
<td>26</td>
<td>ng/ml</td>
<td>nanogram/milliliter</td>
</tr>
<tr>
<td>27</td>
<td>FSH</td>
<td>Follicle Stimulating Hormone</td>
</tr>
<tr>
<td>28</td>
<td>GnRH</td>
<td>Gonadotropin Releasing Hormone</td>
</tr>
<tr>
<td>29</td>
<td>CL</td>
<td>Corpus Luteum</td>
</tr>
<tr>
<td>30</td>
<td>CBC</td>
<td>Complete blood count</td>
</tr>
<tr>
<td></td>
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<td>---</td>
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</tr>
<tr>
<td>31</td>
<td>i/m</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>32</td>
<td>Inj</td>
<td>Injection</td>
</tr>
<tr>
<td>33</td>
<td>I.U.</td>
<td>International Unit</td>
</tr>
<tr>
<td>34</td>
<td>@</td>
<td>At the rate</td>
</tr>
<tr>
<td>35</td>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>36</td>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>37</td>
<td>Vit.</td>
<td>Vitamin</td>
</tr>
<tr>
<td>38</td>
<td>min</td>
<td>Mineral</td>
</tr>
<tr>
<td>39</td>
<td>hrs</td>
<td>Hours</td>
</tr>
<tr>
<td>40</td>
<td>mg/dL</td>
<td>Milligram per deciliter</td>
</tr>
<tr>
<td>41</td>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>42</td>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>43</td>
<td>T. P.</td>
<td>Total protein</td>
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1. INTRODUCTION

Animal Husbandry and dairying play an important role in development of India's economy. The significant economic growth in milk production through dairy farming is an impact of operation flood programme, started in the year 1970. For fulfilment of the objectives of this programme the crossbreeding of indigenous cows on large scale was started. Today the crossbred cows are playing a vital role in the growing economy of Indian dairy industry. Crossbred cows have become an integral part of dairy farming in India due to its certain advantages such as early puberty and maturity, better productivity and comparatively more lactation length. However, on the other side crossbred cows are facing the problems pertaining to their fertility.

Age at puberty and first conception influences lifetime productivity of cattle, which reflects in the number of calving. Delayed puberty and consequently delayed conception is a very important cause of low reproductive efficiency in cattle. It is one of the important challenging problems of a country like India and which is responsible for huge economic losses to dairy farmers by decreasing lifetime milk yield and the number of calves produced by a cow in her lifetime (Chaudhari et al., 2012).

Most of the heifers attain pubertal age and body weight within stipulated time. The crossbred heifer has shown the average age at maturity about 15-18 months located at different agro climatic condition of the country (Behera et al., 1993). A large percent of heifers fail to commence cyclicity due to which there is increase in economic loss of the farmer as the heifers cannot breed early. To solve the problem of delayed puberty and to induce ovarian activity, there is need of external stimulation to initiate the ovarian cyclicity. Many research workers used herbal heat inducer drugs (Singh et al., 2012), chelated mineral mixture (Kumbhar et al., 2012), vitamins and minerals (Jakhir Hussain et al., 2009), plane of nutrition (Sihag, 2008) to induce ovarian activity. Similarly, several hormonal preparations are also available to induce oestrus in cyclic as well as acyclic animals. These include administration of prostaglandin, progesterone, Gonadotropin and Gonadotropin Releasing Hormone (GnRH) or their synthetic analogues, either alone or in various combinations (Chaudhari et al., 2012).
Delayed puberty defeats the objective of cross breeding to have more calf crop and life time milk production. It is due to failure in triggering pituitary function through endogenous hypothalamic releasing factors. Administration of GnRH analogue mimics the sequence of events essential for oestrus manifestation (Dhoble and Gupta, 1986). However, the induced oestruses are mostly silent and hence have a low conception rate.

Among hormone preparations, Gonadotropin-releasing hormone (GnRH) and its analogues cause an acute secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) such that concentrations in peripheral blood are elevated for a 3 to 5 hour period. The mechanism of action of GnRH is via the induction of accessory corpus luteum (CL) by dominant follicle ovulation of the first follicular wave. It had also having a stimulatory effect on the production of progesterone by the primary CL (Schmitt et al., 1996). Thus, GnRH has the property of inducing follicular growth and ovulation.

The introduction of Clomiphene citrate in veterinary profession has been reported as a good tool for treating anoestrus cows and buffaloes by Deshpande et al. (1976), Kaikini et al. (1977), Hukeri et al. (1979), Dugwekar et al. (1980), Chandra et al. (1990).

Clomiphene citrate was previously considered as an anti oestrogen; recently it was reclassified as a selective oestrogen receptor modulator (Shelly et al., 2008). Clomiphene Citrate is known to be both an estrogen agonist and antagonist. However, its agonist properties manifest only when endogenous oestrogen levels are extremely low. In general its antagonistic effects prevail. Clomiphene citrate administration leads to depletion of estrogen receptors at the level of the pituitary and hypothalamus, interrupting the negative feedback that estrogen normally produces. As a result, GnRH secretion is improved and stimulates pituitary production of follicle-stimulating hormone (FSH), which in turn drives follicular growth and maturation with emergence of one or more dominant follicles (Schorge et al., 2008).

Nutrition plays vital role in reproduction. Reproductive performance of farm animals depends on adequate balanced levels of vitamins and essential minerals due to their important roles in cellular metabolism, maintenance and growth (Sweta
et al., 2009). In addition, these nutrients have specific roles and requirements in reproductive tissues. Poor nutrition delays puberty reduces conception rate and increases pregnancy losses in heifers (Lemenager et al., 1980). Drought and underfeeding delays the onset of puberty in heifers and stops ovarian activity in those animals that have already reached puberty (Post and Reich, 1980). Deficiency of trace elements leads to inactive ovaries and repeat breeding in dairy animals. The blood mineral profile during post partum period has great relevance to future fertility in dairy animals. The presence of low or very low mineral status in blood and response to specific mineral element may be helpful in the diagnosis of mineral response disorder. Mineral like calcium and phosphorus play an intermediate role in the action of hormone and enzyme at sub cellular levels in an integrated fashion in the initiation of oestrus in young growing heifers.

Vitamins are essential micronutrients for proper function of various vital organs including genital organs and endocrine glands due to their obvious roles in cellular metabolism, maintenance and growth (Abou El-Ghait et al., 2004). It is observed that, vitamin A is clearly present at the ovarian level and in steroidogenesis. Higher vitamin A concentrations are found in non-atretic follicles and this might indicate a role of vitamin A in follicular development (Schweigert and Zucker, 1988). Vitamin D is responsible for regulation of intra-cellular calcium and calcium binding protein in reproductive organs and pituitary (Smith et al., 2000). Recently immunohistochemical analysis revealed that vitamin D receptors mRNA are expressed in denderetic cells, macrophages and luminal and glandular epithelial cells of the endometrium, granulosa and cumulus oophorus cells of the ovary and fallopian epithelial cells, particularly during oestrus phase suggesting its immunological role during this period (Zarnani et al., 2010). On the other hand, Vitamin E affects reproductive tissue through their antioxidant roles as well as involvement in prostaglandin synthesis (Robinson et al., 2006). Significant low value of calcium and inorganic phosphorous, total protein, albumin, globulin, glucose and cholesterol concentrations were assayed in the serum of delayed pubertal heifer as compared with normal animals (Ryan et al., 1997).

Haematology and biochemical constituents of blood have great diagnostic value in the clinical practice. They also aid in understanding the reasons associated with low calf crop in most of the domestic animals (McClure, 1965; Lamond, 1970;
Awasti and Kharche, 1987). Deficiency of certain blood constituents macro and micro nutrients may cause infertility. Supplementation of the deficient mineral of appropriate salt in proper dose can reduce the incidence of infertility (Arthur (1975) reported that pituitary function may be influenced by blood glucose level.

Despite considerable economic importance associated with factors that control the onset of puberty, still there is wide scope for research on this aspect. Therefore the present research work was carried out with the aim to study the effect of supplementation of Clomiphene citrate, Vitamin A, D₃, E + Phosphorus and GnRH in delayed pubertal crossbred heifers with following objectives.

**Objectives**:

1. To study efficacy of Clomiphene citrate, Vitamin A, D₃, E, sodium 4-dimethylamino-2-methyl-phenyl-phosphonate and GnRH for induction of oestrus in crossbred heifers.
2. To study the oestrus induction period, duration of oestrus, intensity of oestrus and conception rate in different groups of crossbred heifers.
3. To study the comparative haemato-biochemical profile in delayed pubertal crossbred heifers before and after treatment.
2. REVIEW OF LITERATURE

Delayed puberty in crossbred heifers is one of the most important gynaecological problems normally observed in villages. It is a sign of temporary ovarian dysfunction caused by several factors such as, environmental stress, nutritional deficiencies, extreme environmental temperature, etc. These factors are responsible for the delay initiation of ovarian activity. However, delayed puberty condition in crossbred heifer is mostly ascribed to nutritional deficiency and hormonal imbalances. Several therapies are being used to treat the condition of true anoestrus in crossbred heifer with varying degree of success. Therefore the present study “Therapeutic management of delayed puberty in crossbred heifers.” was carried out under field condition in Pune, Nasik and Ahmednagar district and in the Department of Animal Reproduction, Gynaecology and Obstetrics, Bombay Veterinary College, Parel, Mumbai. The relevant scientific literature on these aspects has been reviewed under the following sub-heads.

2.1 Efficacy of Clomiphene citrate in oestrus induction (%), oestrus induction period, duration of oestrus and conception rate.

2.2 Efficacy of Vitamin AD₃E and Tonophosphan in oestrus induction (%), oestrus induction period, duration of oestrus and conception rate.

2.3 Efficacy of GnRH in oestrus induction (%), oestrus induction period, duration of oestrus and conception rate.

2.4 Oestrus intensity score for Clomiphene citrate, AD₃E + Phosphorus and GnRH Treatment.

2.5 Biochemical profile before and after treatment

2.6 Haematological profile before and after treatment
2.1 Efficacy of Clomiphene Citrate for Oestrus Induction (%), Oestrus Induction Period, Duration of Oestrus and Conception Rate.

Deshpande et al. (1976) during the study undertaken for induction of oestrus with Clomiphene citrate treated eighteen post-partum anoestrus cows and nine buffaloes with Fertivet @ 300 mg daily for five days. Nine cows (50%) and all buffaloes (100%) exhibited oestrus within 4 -11 days and 2-8 days, respectively.

Kaikini et al. (1977) treated anoestrus cows with Clomiphene with two different doses; 500 mg and 300 mg each ‘Fertivet’ single dose treatment (Fertivet 500 mg) was found to have only ‘superficial’ effect on congestion of genitalia but their was neither follicular growth nor ovarian activity. While, ‘Fertivet’ five-day treatment (FVT 300 mg) gave most encouraging results as 60 % cases manifested signs of heat. It was concluded that ‘Fertivet-300’ therapy was a near break-through for tackling true anooestrus problem in cattle.

Kodagali (1978) treated 20 anoestrus Gir cows by giving tab. Fertivet with a dose of daily 300mg for 5 days. The drug was administered as a drench following Cuso4 1% solution. He recorded 60 % oestrus induction with 25 % conception rate and 22.42 ± 6.29 days require to induce oestrus.

Kodagali et al. (1978) dosed 4 anoestrus Gir cows (trial I) by giving tab. Fertivet (each tablet containing CIS-Clomiphene citrate 180 mg, 120 mg Trans-Clomiphene citrate) with a dose of daily 300mg for 5 days. The drug was administered as a drench following Cuso4 1% solution. All animals under trial reacted to the administration of Fertivet with positive results. In that trial average oestrus induction after treatment was 3.33 days.

Pargaonkar (1978) recorded 60.00 % of oestrus response in the native and cross bred heifers when treated with Clomiphene citrate,

Hukeri et al. (1979) done trials on 47 anoestrus lactating buffaloes by giving 300mg Fertivet tabs for 5 day to study oestrus induction percentage, average day require to exhibit oestrus and conception rate (%). They found oestrus induction, day require to exhibit oestrus and conception rate was 85.72 %, 11.13 days and 80
% respectively while in control group these values are 50 %, 32.83 days and 50 % respectively.

Dugwekar et al. (1980) treated 5 Brown Swiss, 5 Jersey crossbred and 6 Sahiwal anoestrus cows with Fertivet for 5 days. Out of these, 3 (60 %), 4 (80 %) and 3 (50 %) cows, respectively exhibited oestrus within 5 days after the end of treatment.

Dugwekar et al. (1981) reported the oestrus response of 87.50% in the cows treated with Fertivet (Clomiphene citrate).

Varma and Kharche (1983) conducted a trial on 5 post-partum anoestrus buffaloes were treated with one Fertivet tablet (300 mg) once daily for 5 days with 125 ml of 1% CuSO₄ solution and they found that 3 (60 %) treated buffaloes exhibits oestrus within average 21 days of treatment.

Kurien and Madhavan (1985) treated a total number of 52 (19 cows and 33 heifers) animal with Fertivet. They recorded 63.64 % oestrus induction in heifers and 68.42 % in cows within 4.48 ± 0.21, 4.57 ± 0.16 days of interval in heifers and cows respectively. The conception rate in induced heat was 42.11 % in cows and 30.30 % in heifers when treated with Fertivet.

Deen and Tanwar (1988) studied the efficacy of Clomiphene citrate in 12 seasonal anoestrus in buffaloes. Out of these twelve animals, 6 were given treatment and 6 were kept as control. Four (66.66 %) out of 6 animals ovulated after treatment as compared to none in the control group during the summer season. Further they recorded only one (25 %) out of four are conceived.

Banerjee and Roychoudhary (1989) conducted a clinical trial for induction of oestrus in prolonged post-partum anoestrus Murrah grade buffaloes with various drugs. Out of 20 buffaloes treated with Fetivet, 16 (80 %) manifested heat with a mean interval of five days and with 75 % conception rate.

Sudhirchandra et al. (1990) studied the effect of Clofert-Vet treatment in post-partum anoestrus crossbred cows and buffaloes. Twenty each, cows and buffaloes were treated with Clofert-Vet. whereas, 10 crossbred cows and 10 buffaloes were kept as control. Out of the treated animals, 60 % cows and 85 % buffaloes exhibited ovulatory oestrus with an average interval of 8.42 ± 0.98 and
6.01 ± 0.41 days, respectively. In control group, only 30% cows and 40% buffaloes exhibited oestrus with an average interval of 33.33 ± 3.39 and 26.00 ± 2.35 days, respectively.

Kadu and Chede (1992) evaluated the efficacy of Fertivet (FVT 300) for inducing oestrus in 19 buffaloes during summer. Out of 19 buffaloes, 13 (68.42%) exhibited oestrus, and 84.10% animals were conceived.

Purohit and Bishnoi (1993) reported that 7 cows and 12 heifers were treated with Fertivet. The treatment induced oestrus in 57.10% cows and 83.33% heifers with 71.42% conception rate in heifers and 50% in cow. They further reported that, on an average, heifers required only 5.9 days after end of treatment whereas cows required 8.75 days for induction of oestrus.

Reddy et al. (1994) studied the efficacy of various drugs to induce oestrus in rural post-partum anoestrus buffaloes. Among the non-hormonal drugs tried, Fertivet-induced oestrus in 80.00% of the treated animals with a mean interval of 21 days and 87.50% conception rate to first insemination.

Mathur and Sharma (2007) gave Clomiphene citrate to anoestrus Frieswal heifer and recorded 42.80% oestrus induction.

Kankal et al. (2008) treated six anoestrus crossbred cows with Clomiphene citrate @ 300 mg orally with 125 ml of 1 percent copper sulphate solution prior to the administration of Fertivet. Out of 6 cows, 3 cows (50.00%) exhibited oestrus within 5-8 days of interval. The type of oestrus was intense in one anoestrus cow whereas it was weaker in two cows. One more cow exhibited oestrus; however, ovarian activity was not noticed.

More (2013) studied on eight true anoestrus crossbred cows treated with Clomiphene citrate. She reported 7.67 days require inducing 50% oestrus induction with 60% conception rate.
2.2 Efficacy of Vitamin AD₃E and Tonophosphan for Oestrus Induction (%), Oestrus Induction Period, Duration of estrus and Conception rate.

Kulkarni (1973) did clinical trial of inj. Tonophosphan + prepalin on eleven anoestrus heifers. He took ten anoestrus heifers in control Group and eleven heifers in Group II which were treated with inj. Tonophosphan + prepalin. He observed that 40 % heifers exhibited the first oestrus within 33.25 ± 12.75 days of interval with 20 % conception rate in control Group. Further he observed that 81.81 % heifers exhibited the first oestrus within 28.66 ± 6.7 days of interval with 36.36 % conception rate after treatment in Group II.

Jackson et al. (1981) studied Endocrine and ovarian changes in dairy cattle fed a low beta-carotene diet during an oestrus synchronisation regime. Twelve Friesian cows, 76 to 110 days calved, with blood cell counts and Compton metabolic profile values in the normal range throughout, were housed and fed a marginal diet for varying periods before being injected twice, 11 days apart, with cloprostenol. Artificial insemination was carried out 72 and 96 hours after the second injection. Plasma concentrations of progesterone, oestradiol 17 beta, luteinising hormone, follicle stimulating hormone, and beta-carotene were monitored during this regime as were uterine and ovarian changes. Progesterone profiles were followed for a further 21 days by assay of milk samples. Subsequent rectal examinations showed that five of the 12 cows conceived at controlled oestrus and another four within 52 days of this time. No correlation was observed between time of conception and condition score, metabolic profiles or haematological parameters; there was a correlation between time of conception and plasma beta-carotene concentrations and cows with lower beta-carotene values showed cyclic irregularities or appeared to have depressed steroid hormone production.

Singh and Vadnere (1987) reported 72.22 % oestrus induction in eighteen crossbred anoestrus cows which was treated with deficient mineral supplements.

Baruah et al. (1989) recorded 17.90 ± 0.48 hrs duration of oestrus in anoestrus crossbred heifer after giving beta carotene supplement.

Markandeya et al. (2002) induced oestrus in 20 anoestrus Deoni cows by giving Gynalactin (micro minerals with Vit. E) Supplementation. The oestrus response was 75 % with 23.04 ± 2.18 hrs duration of oestrus. They found 75 days
required to induce oestrus after giving treatment. The Conception rate was 100 % as all animal which came in oestrus conceived.

Mathur et al. (2004) conducted their study on fifteen Frieswal and eight Sahiwal anoestrus heifers. They recorded after giving Tonophosphan + Prepalin, 80% Friezwal heifers and 66.6 % Sahiwal heifers exhibits oestrus after 10.25 and 21 days of treatment respectively. Further they recorded 100% conception rate in both Friezwal and Sahiwal heifers.

Singh et al. (2006) conducted study on ten anoestrus crossbred cattle. They gave injection Urimin 10 ml i/m route (Group II) alternate day for 3 days and they recorded 60 % treated animal showed oestrus induction.

Mathur and Sharma (2007) had given Agrimin fort to anoestrus Frieswal heifers @ 40gm for 10 days and recorded 57 % oestrus induction.

Hussain et al. (2009) studied Comparative response of vitamin-mineral in alleviating post-partum true anoestrus in dairy cows. They recorded 87.5% oestrus induction and 71.4% conception rate after giving Agrimin powder (Glaxo) @ 30 gram orally daily for 21 days and vitamin- A injection (Glaxo) 4 ml i/m on alternate day for 3 days to 24 anoestrus cow.

Kumar et al. (2011) treated 13 true anoestrus cross bred cows with nutritional supplementation like Tonophosphan, Prepalin injection (18 lakhs I.U. Vit. A) and cyclomin plus for 15 days and they found 76.92 % oestrus induction response with mean time interval of oestrus as 26.70 ± 4.52 days. They recorded 40 % conception rate

Ahmed et al. (2012) conducted a trial to ameliorate the reproductive performance of 50 native cows suffering from reduced fertility. They found that after giving Vitamin AD₃E supplementation, 80 % of cows in the treated group showed oestrous signs within 7-14 days of post treatment in the treated than the control groups. After 2 months from treatment, 64 % of cows in the treated groups were found pregnant. They concluded that supplementation of low fertile cows with AD₃E improves the reproductive performance
Bhaskar et al. (2013) studied 10 post partum crossbred cows by giving Vitamin A + Tonophosphan and they found that within 21.17 ± 1.08 days of interval 60 % animal induces oestrus after treatment.

2.3 Efficacy GnRH for Oestrus Induction, Oestrus Induction Period, Duration of Oestrus and Conception Rate

Dhoble and Gupta (1986) treated 53 anoestrus cows of Haryana crossbred with Holstein Friesian, Brown Swiss and Jersey half-bred crosses with 21 µg Gonadotropin releasing hormone intramuscularly. Out of 53, nine animals responded between 4-10 days after the treatment, while three animals took 14-55 days i.e. overall 22.46 % animal showed oestrus induction.

Mujumdar (1989) studied the effect of GnRH analogue 5 ml i/m in anoestrus cows. Out of the 32 treated anoestrus cows, 16 animals i.e. 50 % showed oestrus induction within 8 to 14 days.

Rao and Venkatramiah (1990) studied the induction and synchronization of oestrus and fertility in seasonally anoestrus buffaloes with GnRH analogue. Forty eight female buffaloes were treated with single injection of GnRH @ 100 µg i/m. GnRH induced oestrus in 5 buffaloes (i.e. 10.42 %) within 1-2 days of treatment.

Zaghloul et al. (1993) treated five long acyclic postpartum Jersey cows with GnRH analogue-Fertagyl, 250 mg i/m. Four cows exhibited oestrus within 3 days after treatment. The remaining cows came into oestrus within 25 days after treatment i.e. oestrus induction was 100 % within 3 to 25 days of interval. Rectal examination showed that all the 5 cows were pregnant after 6 weeks (i.e. conception rate was 100 %)

Sonwane et al. (1995) reported that 87.50 % of anoestrus cows treated with 5 ml Receptal i/m exhibited oestrus within 8 to 20 days, with the conception rate of 85.71 %.

Nautiyal et al. (1997) investigated the efficacy of GnRH (1.5 ml vs. 2.5 ml. i/m) administration in inducing oestrus and ovulation in anoestrus pubertal heifers; 85.71 vs. 71.42 % of heifers exhibited oestrus within 3.46 and 2.60 days of injection,
respectively, for the 2 dose regimens. The pregnancy rate for both the treated groups was 80 %.

Dantre et al. (1998) induced oestrus in delayed pubertal crossbred heifers after injecting Receptal. They recorded that 85.71 % heifers expressing oestrus within 23.5 ± 1.89 days post treatment with 66.66 % conception rate.

Singh and Madan (1999) studied the effect of GnRH on plasma FSH and LH in buffaloes and found that the plasma FSH elevated significantly (P<0.05) at 5 min and reached peak level (110.06 ± 23.56 ng/ml) by 90 Minute., the value decline to pre - treatment level by 6 hr after GnRH injection. LH level elevated significantly (P<0.05) by 10 min, reaching peak level of 13.15 ± 3.13 ng/ml (P<0.01) by 90 min and declined to basal level by 4 hr after GnRH injection

Thakur and Bhatt (2001) observed oestrus induction in 7 delayed pubertal crossbred heifers with Receptal preparation. They recorded 23.50 ± 1.98 days of oestrus induction with 66.66 % conception rate in crossbred heifers.

Markandeya and Bharkad (2004) induced oestrus in Murrah buffaloes in early and late winter groups (n=8) with Buserelin-acetate @ 0.21 mg i/m. The oestrus response was 100.00% and 75.00% in early and late winter, respectively. Conception rate and oestrus induction in hrs were 75.00 %, 21.35 ± 1.98 in early winter and 50.00%, 23.69 ± 1.62 in late winter group respectively. They concluded that treatment with GnRH was effective in bringing about fertile oestrus and conception in post partum anoestrus buffaloes.

Khasatiya et al. (2005) studied the effect of GnRH treatment on postpartum true anoestrus (n=6) Surti buffaloes. They recorded Five (88.33 %) GnRH treated true anoestrus buffaloes exhibited heat.

Singh et al. (2006) conducted study on 10 anoestrus crossbred cattle by giving inj. Receptal 5 ml i/m (Group I) and reported 90 % oestrus induction after treatment.

Sirmour et al. (2006) performed trial on 42 delayed pubertal crossbred heifers. Out of which 6 animals were given injection Buserelin acetate 20 mcg intramuscular single dose. They found that oestrus induction efficacy percentage,
oestrus induction interval in a day and conception rate were 83.33%, 12.60 ± 3.00 days and 40 %, respectively.

Bhutani et al. (2009) injected 15 true anoestrus buffaloes with GnRH (Receptal 5.0 ml) i/m, single injection. The oestrus induction response and conception rate was 86.66% and 38.46%, respectively with oestrus induction interval of 21.50 ± 5.49 days.

Ahmed et al. (2010) had done study on normal cyclic and delayed pubertal buffalo heifers. Delayed pubertal Egyptian buffalo heifers (n=37) were intramuscularly injected with 200µg of GnRH (Receptal) and recorded 75.68 % animal showed oestrus induction after treatment.

Kumar et al. (2011) treated 13 true anoestrus cross bred cows with GnRH (Receptal, 5 ml) i/m, single injection and found 84.62% oestrus induction response with mean time interval of oestrus as 28.27±6.46 days and 72.73 % conception rate.

Gupta et al. (2012) conducted studies on 20 post partum anoestrus cows in terms of oestrus induction, efficacy, oestrus induction interval and conception rate. Out of total 20 animals, 10 animals were given GnRH injection, they found that 50% animals showed oestrus induction with 10.16 ± 0.33 days of oestrus interval period and 80% overall conception rate.

More (2013) treated 8 true anoestrus crossbred cows which treated with injection of 5 ml Receptal (Buserelin-acetate, an analogue of Gonadotropin releasing hormone) @ 0.02 mg (total single dose) intramuscularly. She reported 9.80 days require to induce 62.50 % oestrus induction with 83.33 % conception rate after first dose of Receptal.
2.4 Oestrus Intensity Score for Clomiphene Citrate, AD$_3$E + Phosphorus and GnRH Treatment

Baruah et al. (1989) recorded 78.57 % heifers showed pronounced oestrus intensity and 21.43 % heifers with weak intensity in anoestrus crossbred heifer on giving beta carotene supplement.

Sirmour et al. (2006) studied 42 delayed pubertal crossbred heifers. Out of which 6 animals were given injection Buserelin acetate 20 mcg intramuscular single dose and they recorded 77.40 ± 4.01 estrus intensity score after treatment.

Garcia et al. (2011) observed the relationship between intensity of oestrus signalling and reproductive performance as pregnancy and calving rate in cows and heifers. In present study 75 % heifers showed high Intensity of Oestrus and pregnancy and calving rate was 80 and 75 % respectively. Whereas 67 % heifers showing low pregnancy rate 45 % and calving rate 39 % respectively. They concluded that the higher intensity of oestrus and the higher the pregnancy and calving rates.

Ahmed et al. (2012) conducted a trial to ameliorate the reproductive performance of 50 native cows suffering from reduced fertility. They found that after giving Vitamin AD$_3$E supplementation oestrous signs were more intense (3.46 ± 1.33) in the treated than the control groups.

Rao et al. (2013) had used following technique to score oestrus intensity.

Score 1: Nervousness and unusual interest in herd mate, nutritional status of animals with thin glary vaginal discharge.

Score 2: Period of intense heat with more vaginal discharge, considerable excitement and mounting of other females.

Score 3: Standing to ridden with or without the symptoms given for score 1 or 2.
2.5 Biochemical Profile Before and After Treatment

2.5.1 Serum calcium

Prasad et al. (1984) reported serum calcium level in anoestrus crossbred cow as 9.82 ± 0.36 mg/dL before treatment and 10.05 ± 0.39 mg/dL after treatment with Fertivet.

Hafez (1987) observed that the highest level of serum calcium in the induced estrus cow might be due to increased level of estrogen which mobilized calcium from different body depots to blood that is necessary for genital tract contraction.

Bagal and Kadu et al. (1988) reported insignificant (P<0.05) difference in serum calcium value in Control Group 9.19 ± 0.49 and 9.48 ± 0.57 mg/dL in treated Group after giving Receptal treatment to eighteen post partum anoestrus crossbred cow.

Singh and Vadnere (1989) recorded blood plasma level of Calcium in eighteen anoestrus crossbred cows after supplementation of deficient minerals. They found values of calcium were 9.667 ± 0.25 mg/dL before treatment and 12.420 ± 0.28 mg/dL at the time of oestrus with statistically significant difference (P<0.01)

Lazar et al. (1992) during the study on calcium signalling and episodic secretion of GnRH stated that the pulse of GnRH could be abolished in Ca\(^{2+}\) deficient medium. It was demonstrated that pulsatile neuropeptide secretion was an intrinsic property of GnRH neuronal networks and was dependent on Ca\(^{2+}\) influx for its maintenance.

Behera et al. (1993) reported insignificant difference (P<0.05) between pre treatment (10.01 ± 0.10 mg/dL) and post treatment serum calcium values (10.27 ± 0.11 mg/dL) in delayed pubertal cross bred heifers which was treated with Tonophosphan and mineral mixture.

Purohit and Bishnoi (1993) studied blood serum Calcium levels in anoestrus cow (group I), Fertivet induced oestrus cows (group II) and normal oestrus cows (control group). Calcium values were significantly higher in Fertivet induced oestrus group (11.30 ± 0.825 mg/dL) and normal oestrus group (10.95 ± 0.647 mg/dL) as
compared to anoestrus group (10.47 ± 0.904 mg/dL). However, non-significant difference was observed between group II and control group animals.

Dantre et al. (1998) induced oestrus in delayed pubertal crossbred heifers with GnRH. They reported the serum Calcium level in normal cycling heifers was 10.39 ± 0.78 mg/dL and in delayed pubertal heifers it was 9.59 ± 0.79 mg/dL. The values did not vary significantly. But the GnRH treated heifers showed significant difference (P<0.05) in serum Calcium levels than that in pre-treatment state.

Singh et al. (2006) conducted study on 20 anoestrus crossbred cattle. They gave injection Receptal 5 ml i/m single to 10 animals (Group I) and injection Urimin 10 i/m (Group II) alternate day for 3 days to 10 animals. The calcium levels at 0 day in Group I was 9.36 ± 0.44 mg/dL and in Group II was 8.63 ± 0.31 mg/dL. At the time of oestrus induction these values were 10.19 ± 0.40 and 9.67 ± 0.29 mg/dL for Group I and Group II respectively.

Ahmed et al. (2010) had done study on normal cyclic and delayed pubertal Egyptian buffalo heifers. Delayed pubertal Egyptian buffalo heifers (n=37) were intramuscularly injected with 200µg of GnRH (Receptal) and response were recorded. Result indicated that there is significant difference (P<0.001) in serum calcium value in normally cyclic (10.23 ± 0.21) and delayed pubertal buffalo heifer (9.07 ± 0.10).

More (2013) studied 16 true anoestrus crossbred cows Group I (n=8) were treated with injection of 5 ml Receptal and Group II (n=8) were treated with bolus of Fertivet containing Clomiphene citrate @ 300 mg orally per day for five days. She recorded serum calcium values before treatment were 8.41±0.27 mg/dL in Group I and 8.51 ± 0.46 mg/dL for Group II animals. At the onset of oestrus she reported serum calcium values 9.18 ± 0.30 and 8.56 ± 0.29 mg/dL in Group I and Group II animals, respectively.
2.5.2 Inorganic phosphorus

Maynard et al. (1979) concluded that commonly available dry roughages mainly paddy straw which contain low level of most of the micronutrient except iron. Phosphorus content in most of the grasses was usually deficient which might due to the fact that phosphorus contain in soil was deficient as well as high contain of iron in the soil render the phosphorus as insoluble phosphate.

Prasad et al. (1984) reported serum inorganic phosphorus level in 10 anoestrus crossbred cow was $7.33 \pm 0.63$ mg/dL before treatment and $7.59 \pm 0.61$ mg/dL after treatment with tablet Fertivet which was with statistically significant difference ($P<0.05$).

Chandolia et al. (1987) conducted study on 32 Murrah buffalo heifers of three and half to four years of age and recorded that the mean plasma inorganic phosphorus level was significantly higher ($4.58 \pm 0.39$ and $4.73 \pm 0.34$ mg/dL) in cycling heifers than anoestrus heifers ($3.18 \pm 0.15$ mg/dL).

Singh and Vadnere (1987) had reported serum inorganic phosphorus $6.22 \pm 0.02$ mg/dL before treatment and $7.19 \pm 0.15$ mg/dL after treatment in 18 cross bred anoestrus cows which was treated with deficient mineral supplements.

Behera et al. (1993) reported significant difference ($P<0.01$) between pre treatment ($3.03 \pm 0.12$ mg/dL) and post treatment of serum inorganic phosphorus values ($5.26 \pm 0.12$ mg/dL) in delayed pubertal cross bred heifers which was treated with mineral mixture and Tonophosphan.

Purohit and Bishnoi (1993) as they recorded higher ($5.65 \pm 0.66$ mg/dL) but non-significant serum inorganic phosphorus level in anoestrus cows induced for oestrus with Fertivet over the ($5.24 \pm 0.89$ mg/dL) pre-treatment value.

Dantre et al. (1998) induced oestrus in delayed pubertal crossbred heifers with GnRH therapy. They reported that the serum phosphorus level in normal cycling heifers were higher ($5.59 \pm 0.47$ mg/dL) than that in delayed pubertal heifers ($4.63 \pm 0.43$ mg/dL). In GnRH treated group, the pre-treatment levels ($4.59 \pm 0.24$ mg/dL) and post-treatment levels ($5.06 \pm 0.23$ mg/dL) differed significantly ($P<0.05$).
Dutta et al. (2001) reported the significant increase in the calcium and inorganic phosphorus level in heifers after the treatment and in induced oestrus it may be due to supplementation of mineral mixture before giving treatment or due to fluctuating levels of estrogen in oestrus and the lower levels of serum calcium in delayed pubertal heifers. This might be due to failure of endocrine system to mobilize the body calcium which leads to reproductive failure.

Yadav et al. (2004) conducted study on non-cyclic and cyclic crossbred cows detected in oestrus on 30 days postpartum and recorded the values of inorganic phosphorus as 4.48 ± 0.063 to 5.97 ± 1.93 mg/dL respectively. Significant difference (P<0.05) was found in inorganic phosphorus between cyclic and non cyclic crossbred cows.

Singh et al. (2006) performed study on 20 anoestrus crossbred cattle. They gave injection Receptal 5 ml i/m single to 10 animals (Group I) and injection Urimin 10 i/m (Group II) alternate day for 3 days to 10 animals. The phosphorus levels at 0 day in Group I was 5.03 ± 0.26 mg/dL and for Group II it was 4.84 ± 0.24 mg/dL. At the time of oestrus induction these values were 6.68 ± 0.40 mg/dL for Group I and for Group II 8.34 ± 0.16 mg/dL respectively.

2.5.3 Glucose

Kulkarni (1973) reported glucose level 57.83 ± 7 and 64.25 ± 7.33 mg/dL in control and Tonophosphan + prepalin treated anoestrus heifers.

Agarwal et al. (1985) studied blood glucose level in normal cyclic and anoestrus cases. Blood glucose level were significantly lower in anoestrus animals (62.90 ± 10.55 mg/dL) as compared to normal cyclic (84.54 ± 13.37 mg/dL) crossbred cows.

Mc Cann and Hansel (1986) found that the aberrant pituitary and luteal functions in fasted heifers were associated with concurrent fasting-induced changes in insulin and glucose metabolism.

Shrivastava and Kharche (1986) estimated serum glucose levels in normal cycling and anoestrus buffaloes. They observed that the average serum glucose levels were 54.62 and 69.42 mg/dL, respectively in anoestrus and on the day of
oestrus after giving treatment with Fertivet in buffaloes and concluded that glucose levels were significantly lower during anoestrus condition.

Bagal and Kadu (1988) recorded non significant difference in the blood glucose levels of postpartum crossbred cows treated with GnRH (45.17 ± 2.43 mg/dL) and non-treated cows (48.57 ± 1.15 mg/dL) during first postpartum ovulation and also at detected oestrus.

Purohit and Bishnoi (1993) they recorded non-significant difference in the levels of serum glucose before treatment (44.27 ± 3.26 mg/dL) and after treatment (46.07 ± 5.73 mg/dL) in the heifers treated with Fertivet.

Ramkrishna (1997) observed significantly low glucose levels (49.09 ± 12.03 mg/dL) in anoestrus crossbred cows as compared to cycling cows (62.20 ± 3.23 mg/dL).

Dantre et al. (1998) induced oestrus in delayed pubertal crossbred heifers using GnRH. They reported that blood glucose level in normal cycling heifer was 50.59 ± 2.53 mg/dL and that in delayed pubertal heifers was 47.57 ± 2.28 mg/dL. The values between two groups did not vary significantly. But in the GnRH treated group the pre and post treatment glucose levels differed significantly (P<0.05).

Singh and Singh (2005) recorded glucose value in anoestrus crossbred heifer’s 41.01 ± 1.91 mg/dL and in cyclic heifers at the time of oestrus were 56.31 ± 4.04 mg/dL with significant difference (P<0.01)

More (2013) observed significant difference in the levels of serum glucose before treatment (49.11 ± 1.74 mg/dL) and after treatment (60.12 ± 2.17 mg/dL) in the post partum anoestrus cows which was treated with Receptal. Further she recorded non-significant difference in the levels of serum glucose before treatment (54.72 ± 2.76 mg/dL) and after treatment (61.52 ± 3.28 mg/dL) in the post partum anoestrus cows which was treated with Fertivet.

### 2.5.4 Total Protein, Albumin and Globulin

Bagal and Kadu et al. (1988) reported non significant (P<0.05) difference in total protein value in anoestrus Group (6.74 ± 0.13 vs. 6.59 ± 0.11 gm/dL) and in
treated Group after giving Receptal treatment to eighteen post partum anoestrus crossbred cow.

Kavani et al. (1987) emphasized that low level of serum protein influenced the reproductive status in heifers. The lower level of total serum protein may cause deficiency of particular amino acids required for synthesis of various releasing hormone and pituitary hormone causing in turn reproductive disturbances. Protein being the building blocks of the body lack of sufficient protein intake has been regarded as one of the causes of failure of or delay in resumption of oestrous cycle mainly due to retarded development of sex organ.

Gujar et al. (1990) studied haematological and blood biochemical profiles of fertile and non fertile oestrus in Kankrej heifers and recorded total protein value 6.34 ± 0.12 gm/dL in non fertile and 7.59 ± 0.09 gm/dL in fertile heifers respectively.

Behera et al. (1993) recorded significant (P<0.05) mean total protein value in pre treated Group and post treated Group which was 6.62 ± 0.17 and 7.20 ± 0.12 gm/dL in crossbred heifer after treating with Tonophosphan + mineral supplements.

Purohit and Bishnoi (1993) studied blood serum total protein levels in, anoestrus Rathí cow and heifers (group I), Fertivet induced oestrus cows (group II) and normal oestrus cows (control group). Total protein values were higher in normal oestrus group (7.52 ± 1.290 gm/dL) and Fertivet induced oestrus group (6.26 ± 0.725 gm/dL) as compared to anoestrus group (5.99 ± 0.79 gm/dL). However, non-significant difference was observed in group II when compared with anoestrus group I animals.

Vhora et al. (1995) reported that the low level of serum total protein in anoestrus condition might cause deficiency of certain amino acids required for synthesis of Gonadotropin.

Hafez and Hafez, (2000) noted the difference in total protein values in estrus heifers, which might be due to the higher intake of protein and non protein nitrogenous substances associated with higher content of energy density in oestrus heifers.

Yadav et al. (2004) conducted study on non-cyclic and cyclic crossbred cows detected in oestrus on 30 days and recorded the values of total protein as 6.18 ±
0.18 to 7.30 ± 0.24 gm/dL. The observation revealed that significant difference (P<0.05) was found in total protein value between cyclic and non cyclic crossbred cows.

Khasatiya *et al.* (2005) studied the Effect of GnRH treatment on conception rate and blood biochemical and mineral profile of postpartum true anoestrus (n=6) Surti buffaloes. They recorded significant difference (P<0.01) in the levels of serum total protein in true anoestrus and Control Group (7.70 ± 0.13 and 7.01 ± 0.21 gm/dL) buffaloes.

Singh and Singh (2005) recorded total protein, Globulin value in delayed pubertal crossbred heifer’s 5.83 ± 0.13, 3.72 ± 0.10 gm/dL and in cyclic heifers at the time of oestrus 6.22 ± 0.11, 3.36 ± 0.12 gm/dL with statistical significant difference (P<0.05). Further they recorded albumin value in delayed pubertal 2.11 ± 0.05 and 2.82 ± 0.08 gm/dL in cyclic heifers at the time of oestrus with significant difference (P<0.01)

Patel and Dhami (2006) studied postpartum plasma profile of glucose in 6 anoestrus Holstein Friesian cows after Receptal therapy under tropical climate. They recorded plasma total protein 6.59 ± 0.11 gm/dL in treated anoestrus cow and 6.74 ± 0.09 gm/dL in control Group of animal. They found plasma glucose value lower in treated anoestrus animal than control group.

Ahmed *et al.* (2010) had done study on normal cyclic and delayed pubertal Egyptian buffalo heifers. 37 delayed pubertal Egyptian buffalo heifers were intramuscularly injected with 200µg of GnRH (Receptal) and response were recorded. Result indicated that there is significant difference (P<0.001) in serum total protein value in normally cyclic (5.33 ± 0.09 gm/dL) and delayed pubertal buffalo heifer (4.78 ± 0.11 gm/dL). They also found significant difference in Albumin (P<0.001) and globulin (P<0.01) value in normally cyclic (3.67 ± 0.05 and 1.67 ± 0.09 gm/dL) and delayed pubertal buffalo heifer (3.41 ± 0.03 and 1.19 ± 0.09 gm/dL) respectively.
2.6 Haematological Profile Before and After Treatment

Shrivastava and Kharche (1986) studied the haematological finding after treatment with Fertivet in buffaloes and they observed that there was significant increase in the Hb, PCV, MCH and Monocyte. They were found significant difference (P<0.05) in Hb level i.e. 7.54 gm/dL before treatment and 7.78 gm/dL on the day of oestrus after treatment of Fertivet.

Ahmed et al. (1993) Were performed investigations to determine I) Cyclic changes in uterine immune response of buffalo-cows that could be responsible for increased susceptibility of the dioestrus uterus to infection and The effect of some genital disorders on the immune response. The number and phagocytic capacity of uterine leucocytes as well as the immunoglobulin contents revealed significant cyclic variations. These parameters were obviously decreased in the uteri of buffalo with bilateral smooth inactive ovaries. On the other hand, marked increases were recorded in both uterine Leukocyte counts and immunoglobulin contents in the uteri of buffalo with cystic ovarian disease, endometritis, pyometra and cervical retention cysts. Phagocytic capacity showed obvious increase in case of cystic ovarian disease while decreased in other studied disorders.

Pradhan et al. (1995) recorded significant increase in Hb value after giving mineral supplements in post partum anoestrus cow. They recorded Hb level 9.57 ± 0.52 dm/dL in post partum anoestrus cow and 11.74 ± 0.60 gm/dL in cyclic cow.

Pradhan et al. (1995) found high numerical difference in Hb value in between pre treated and post treated heifers. This concludes that lower level of haemoglobin might be representing some systemic disarrangement due to deficiencies of certain trace minerals which in turn has depressed the physiological reproduction. Though the important of the level of haemoglobin has not been directly implicated in reproductive disorders, yet the decrease in its value is indicative of certain systemic disorder which can indirectly affect the functional activity of the reproductive organ.

Mallard et al. (1998) Discrepancies in values for various haematological parameters due to differences in sampling interval, methods used number of cows sampled, and / or degree of metabolic disturbances. Moreover, genetic disturbances between heifers.
Victore et al. (2000) reported increase in WBC count after PRID treatment in anoestrus H.F. cows.

Jabbar (2004) observed decreased TLC value in delayed pubertal heifers after treatment. This might be due migration of leucocytes to be infiltrated in tissue of genital tracks especially in cases associated with bacterial infection or unsuitable agro climatic condition.

Meglia et al. (2005) Discrepancies in values for various haematological parameters may involve variation in subtropical condition, management, feeding and changes in hormonal level.

Abdel – Mohsen et al. (2013) reported increase in Hb, RBC and MCV after giving GnRH in crossbred cow.
3. MATERIAL AND METHODS

The present study “Therapeutic management of delayed puberty in crossbred heifers.” was carried out under field condition in Pune, Nasik and Ahmednagar district of Maharashtra state and in the Department of Animal Reproduction, Gynaecology and Obstetrics, Bombay Veterinary College, Parel, Mumbai – 400012 from December 2013 to July 2014.

3.1 Selection of animals

Present research work constituted 32 healthy delayed pubertal crossbred heifers (H.F. X Jersey and H.F. X Gir) devoid of any reproductive disorders, ageing above 19 months and having average body weight between 250-275Kg and above.

3.2 Screening of animals

For present study, delayed pubertal heifers with smooth ovaries were selected. All the animals were subjected to per-rectal examination for ovarian massage at weekly interval. After 3 ovarian massages those heifers which did not show any oestrus signs, having smooth ovaries and free from any reproductive abnormality were taken for the present study. The age of selected heifers were considered by the owner’s history.

The weight of animal was calculated by using Shaffer’s formula as below,

\[ \text{Body weight (pound)} = \frac{(\text{Heart girth})^2 \times \text{Length of the animal}}{300} \]

Where,

1 pound = 0.454 kg

3.3 Deworming and supplementation

Selected crossbred heifers were dewormed with Albendazole @ 10mg/kg body weight orally once before 20 days of inception the study and were supplemented by mineral mixture @ 50gm/animal/day orally for 20 days after deworming.
3.4 Treatment protocol

After deworming and mineral supplementation, all selected delayed pubertal crossbred heifers were randomly divided into 4 groups. Each group consisting of 8 delayed pubertal crossbred heifers. The detail treatment given to each group was as follows.

**Group I** = Heifers in this group were treated with Clomiphene citrate (Ovulanta-Kit manufactured by Vet Mankind Pharma Limited, New Delhi-110 020) @300mg orally for 5 days. Before giving the drug 125 ml 1% CuSO$_4$ of solution was given orally.

**Group II** = Heifers in this group were treated with Inj. Vitamin A @50 lakhs I.U. + Vitamin D$_3$ @ 5 lakhs I.U. + Vitamin E @500 I.U. (Inj.Vetade, manufactured by Zydus A H, Ahemadabad-380 015) I/m and Inj. sodium dimethylamino - 2 – methyl – phenyl – phosphonate @ 2gm, (Inj.Tonophosphate, manufactured by MSD Animal Health) I/m per week for successive 3 week.

**Group III** = Heifers in this group were treated with Inj. GnRH @ 20µg (Receptal® VET, manufactured by MSD Animal Health) I/m as a single dose.

**Group IV** = The heifers of this group were kept as untreated control.

3.5 Post treatment observation

All Crossbred heifers were observed upto 60 days post treatment. Those heifers which responded to the treatment were observed for duration of oestrus induction (days), duration of oestrus (Hrs.) and intensity of oestrus was scored as described by Rao et al. (2012). The heifers showing oestrus were inseminated by using frozen thawed semen. After insemination the heifers were observed for next oestrus. Pregnancy was confirmed by per rectal examination after 2 months of Artificial Insemination.
3.6 **Blood and serum collections**

Blood sample were collected before treatment, on the day of oestrus from delayed pubertal heifers and after 60 days of treatment in those animals which do not exhibit oestrus signs. Blood was collected aseptically from Jugular vein, in EDTA vial for CBC estimation, in sodium fluride vial for glucose estimation and in plane vacutainer vial for serum separation. The serum was separated by allowing blood to clot at room temperature and it was centrifuged to separate serum. Separated serum from each heifer was siphoned out and divided into two equal halves and placed in separate tubes. Each sample tube was promptly named and stored at -20°C till used for biochemical analysis. From collected serum, inorganic phosphorus was estimated within four days of sample collection. The serum calcium, inorganic phosphorus, total protein, albumin, and globulin concentration were estimated using biochemical kit manufactured by ROBONIK INDIA PVT. LTD, Navi Mumbai-400710, India. While CBC was estimated by analyser machine.

3.7 **Statistical analysis**

The recorded data was organised systematically and analysed statistically by using suitable design to draw appropriate conclusion (Snedecor and Cochran, 1994).
4. RESULTS AND DISCUSSION

Delayed Puberty defeats the objective of crossbreeding to have more calf crop and life time milk production. It is due to failure in triggering pituitary function through endogenous hypothalamic releasing factors. Administration of GnRH and Clomiphene citrate mimics the sequence of events essential for oestrus manifestation. Therefore the present study was envisaged to compare the efficacy of GnRH, Clomiphene citrate and vitamin AD₃E supplementation for induction of oestrus and to see that whether it is possible to have better oestrus intensity and conception rate and their effect on the biochemical and haematological parameters.

In present study, 8 delayed pubertal crossbred heifers of Group I were drenched with bolus containing Clomiphene citrate @ 300 mg per day for five days. Before giving the drug 125 ml, 1 % CuSO₄ solution was given orally. In Group II eight delayed pubertal crossbred were treated with combination of injection of Vitamin A (50 lakhs I.U.) + Vitamin D₃ (5 lakhs I.U.) + Vitamin E (500 I.U.) and Inj. of sodium dimethylamino-2-methyl-phenyl-phosphonate - 2 gm were given i/m route per week for successive 3 week as per the prescribed dose of manufacturer. Similarly heifers in Group III were treated with injection of 5 ml GnRH (Buserelin-acetate, an analogue of Gonadotropin releasing hormone) @ 20µg (total single dose) with i/m route once only. Animals from Group IV were kept as untreated. The animals from different II Groups were observed for efficacy of oestrus induction, oestrus induction period, duration of oestrus, intensity of oestrus (score) and conception rate in delayed pubertal crossbred heifers.

The aseptically collected blood and serum (before treatment and on the day of oestrus induction) samples were estimated for CBC, levels of glucose, total Protein (albumin, globulin), calcium and inorganic phosphorus by auto analyzer methods. If the delayed pubertal heifer did not show any oestrus sign during the entire period of experiment then blood was collected at 60th day after completion of treatment for haematological and biochemical analysis. The various detail results of different parameters obtained in the present study were given as follow
4.1 Efficacy of Oestrus Induction

The data pertaining to the rate of induction of oestrus is presented in Table 1. It reveals that 6 out of 8 heifers from Group I exhibited oestrus signs, whereas in Group II, 5 out of 8 heifers exhibited oestrus signs, while in Group III, 7 out of 8 heifers exhibited oestrus and no heifer from Control Group showed any oestrus sign during the entire period of experiment.

### Table 1. Efficacy of oestrus induction in three different Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Efficacy of oestrus induction (%)</th>
<th>Conception rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>6 (75 %)</td>
<td>2 (33.33 %)</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>5 (62.50 %)</td>
<td>4 (80 %)</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>7(87.50 %)</td>
<td>6 (85.71 %)</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>0 %</td>
<td>0 %</td>
</tr>
</tbody>
</table>

The efficacy of oestrus induction in present study for Group I, II, III and IV were 75, 62.50, 87.50 and 0 % respectively. From present study it was found that efficacy percentage was higher in Group III, followed by Group I and then Group II, whereas animal from control Group did not responded to treatment of oestrus induction.

The rate of oestrus induction percentages recorded pertaining to Clomiphene citrate in present findings were higher than the observation recorded by Pargaonkar (1978), Kurien and Madhavan (1985) and Mathur and Sharma (2007) who recorded 60.00, 63.64 and 42.80% oestrus induction in delayed pubertal heifers respectively. Whereas Deshpande et al. (1976), Kaikini et al. (1977), Kodagali (1978), Kurien and Madhavan (1985), Sudhirchandra et al. (1990), Purohit et al. (1993), Kankal et al. (2008) and More (2013) recorded 50.00, 60.00, 60.00, 68.0, 42.0, 60.0, 57.10, 50 and 50 %, oestrus induction in anoestrus cow respectively. Similarly Dugwekar et al. (1980) reported 60 and 50 % estrus induction in Brown Swiss and Sahiwal anoestrus cow respectively. Varma and Kharche

The present findings in Group I were less than the observations recorded by Purohit and Bishnoi et al. (1993). They recorded 83.33 % oestrus induction in delayed pubertal crossbred heifers after giving Fertivet. Whereas, Dugwekar et al. (1980) and Dugwekar et al. (1981), and recorded 80, 87.50 % oestrus induction in anoestrus crossbred cows after giving Fertivet. While Deshpande et al. (1976), Hukeri et al. (1979), Banerjee and Roychoudhary (1989), Sudhirchandra et al. (1990) and Reddy et al. (1994) recorded 100, 85.72, 80, 85 and 80 % estrus induction in buffaloes after giving Clomiphene citrate treatment.

Clomiphene citrate is beneficial in inducing heat and ovulation in anoestrus and repeat breeding cows, heifers and buffaloes it became popular among veterinarians. There is a lot of similarity in the structure of Clomiphene and estrogen. This makes the feedback receptor in the brain to receive Clomiphene instead of estrogen which makes the brain incapable of identifying the normal levels of estrogen. The Clomiphene inhibits estrogen receptors in hypothalamus inhibiting negative feedback of estrogen on Gonadotropin release leading to up regulation of the hypothalamic-pituitary-gonadal axis. Since estrogen no longer effectively exert negative feedback on the hypothalamus, GnRH secretion becomes more rapidly pulsatile which results in increased pituitary Gonadotropin (FSH and LH) release. After administration of Clomiphene, FSH level rise steadily resulting in to the development of new follicles. These follicles in turn produce the estrogen which circulates in the blood and thus the onset of oestrus takes place.

In present study the response of delayed pubertal crossbred heifers to Clomiphene citrate with respect to onset of oestrus was found to be optimum when compared with the observations of earlier studies. Thus, it is opined that Clomiphene citrate is effective in inducing oestrus in delayed pubertal crossbred cows. These results indicate that though the treatment regimen of Clomiphene citrate had good oestrus induction efficacy but had poor conception rate. It might be due to lower ovulatory response and individual variations.

In present study Group II treated with vitamin AD₃E + Phosphorus showed 62.50% oestrus induction in heifers. The lower oestrus induction percentage than
the present study was recorded by Mathur and Sharma (2007) who recorded 57 % oestrus induction in anoestrus Frieswal heifers and for crossbred cow Singh et al. (2006) and Bhaskar et al. (2013) recorded 60 and 60 % oestrus induction.

Whereas higher values than the present study were recorded by Kulkarni et al. (1973) who found 81.81 % oestrus induction in heifers. Similarly Mathur et al. (2004) recorded 80 % oestrus induction in Frieswal heifers and 66.6 % in Sahiwal heifers respectively after giving Tonophosphan + Prepalin. While Singh and Vadnere (1987), Markandeya et al. (2002), Hussain et al. (2009), Kumar et al. (2011) and Ahmed et al. (2012) recorded 72.22, 75.00, 87.5, 76.92 and 80.00 % oestrus induction in cows respectively.

The rate of induction of oestrus recorded pertaining Group III in present findings were found less than the observation recorded by Zaghloul et al. (1994) and Singh et al. (2006) who recorded 100 % and 90 % oestrus induction rate in crossbred cattle. The lower values than the present study were recorded by Nautiyal et al. (1997) reported 85.71 % vs. 71.42 % oestrus induction in 1.5 ml and 2.5 ml dose of GnRH in heifers respectively. Whereas Dantre et al. (1998) and Sirmour et al. (2006) recorded 85.71 % and 83.33 % oestrus induction rate in delayed pubertal crossbred heifers. Mujumdar (1989), Kumar et al. (2011) Gupta et al. (2012) and More (2013) recorded 50, 84.62, 50 and 62.5 % oestrus induction in anoestrus cows respectively. Work done in buffalo revealed that less response than the present study was recorded by Rao and Venkatramiah (1990), Markandeya and Bharkad (2004), Bhutani et al. (2009) and Ahmed et al. (2010), recorded oestrus induction response in true anoestrus buffaloes were 10.42, 75, 86.66 and 75.68 % respectively and high response than present study 100 % and 88.33 % was reported by Markandeya and Bharkad (2004) and Khasatiya et al. (2005) in anoestrus buffaloes.

The rate of induction of oestrus recorded pertaining Group III in present findings were found similar to Sonwane et al. (1995) reported 87.50 % estrus induction in anoestrus cows treated with Receptal.

GnRH is a neurohormonal substance secreted by the hypothalamus and transported by the way of hypophyseal portal circulation to the anterior pituitary, where it controls the production and secretion of follicle stimulating hormone (FSH)
and luteinizing hormone (LH). Systemic administration of Gonadotropin releasing hormone in cattle induces a surge release of both LH and FSH from the anterior pituitary gland causing rapid elevation in plasma concentration to a peak in 60 to 90 minutes and return to pre-injection levels within 4 hrs (Singh and Madan, 1999) simulating endogenous LH and FSH surge that occurs during oestrus in cyclic cows. The FSH stimulates the recruitment and development of new follicles which in turn releases the estrogen in blood circulation.

The oestrus response to the Gonadotropin releasing hormone in present study was found to be less than the finding reported by Markandeya and Bharkad (2004) and Khasatiya et al. (2005). The lesser response may be attributed to high environmental temperature as the study was undertaken in summer month and might be due to poor managemental practices by farmers.

After reviewing the oestrus induction efficacy of Group II and Control Group, the findings suggested that specific vitamin AD$_3$E and Mineral supplementation should be the part of treatment of delayed pubertal crossbred heifers. The present findings were based on a smaller number of animals, but still it be may concluded that the possible improvement in reproduction of heifer can be done by supplementing vitamin AD$_3$E + phosphorus in rice and sugarcane cultivated area where paddy straw and sugarcane tops were feed during green fodder scarcity.

On comparison, the efficacy of these three treatments for induction of oestrus in delayed pubertal heifers, it was evident that the percentage of heifers showing ovulatory oestrus was significantly higher (87.50 %) when treated with GnRH than Clomiphene citrate (75.00 %) and supplemented with Vitamin AD$_3$E and Phosphorus (62.50 %). Thus it could be inferred that all the three treatment were effective in induction of oestrus in delayed pubertal heifers but it appears that GnRH is superior in inducing oestrus with satisfactory fertility.
4.2 Oestrus Induction in Days

The mean time interval recorded for oestrus induction in delayed pubertal crossbred heifers is presented in Table 2. Mean time interval from treatment to induction of oestrus in Group I (Clomiphene citrate) was 37.16 days (range 14 to 50 days). In Group II, treated with Vitamin AD₃E + Phosphorus took mean interval of oestrus induction was 22.20 days (range 7 to 53 days). In Group III treated with GnRH recorded mean interval of oestrus induction 24.43 days (range 6 to 55 days). However, no heifer from Group IV (Control Group) exhibited oestrus.

Table 2. Mean time interval for induction oestrus in different Groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean time interval for induction of oestrus in days (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Clomiphene citrate)</td>
<td>37.16 (14 - 50)</td>
</tr>
<tr>
<td>II (Vit. + min)</td>
<td>22.20 (7 - 53)</td>
</tr>
<tr>
<td>III (GnRH)</td>
<td>24.43 (6 - 55)</td>
</tr>
<tr>
<td>IV (Control)</td>
<td>-</td>
</tr>
</tbody>
</table>

The present findings of oestrus induction days after giving Clomiphene citrate were higher than the days recorded by Kurien and Madhavan (1985) and Purohit and Bishnoi (1993) they recorded oestrus induction 4.48 ± 0.21 and 5.9 days respectively in heifers treated with Fertivet. Lesser days of oestrus induction than the present study were reported by Deshpande et al. (1976), Kodagali (1978), Kodagali et al. (1978) Dugwekar et al. (1980), Kurien and Madhavan (1985), Sudhirchandra et al. (1990), Purohit and Bishnoi (1993), Kankal et al. (2008) and More (2013), they recorded 4 to 11, 22.42 ± 6.29, 3.33, 5, 4.57 ± 0.16, 8.42 ± 0.98, 8.75, 5-8 and 7.67 days respectively for anoestrus cows. While in buffaloes Deshpande et al. (1976), Hukeri et al. (1979), Varma and Kharche (1983), Banerjee and Roychoudhary (1989), Sudhirchandra et al. (1990), and Reddy et al. (1994) recorded 2 to 8, 11.13, 21, 5, 6.01 ± 0.41 and 21 days respectively require to induce oestrus.
The present findings of Group II recorded in the present study were higher than the observations recorded by Mathur et al. (2004), they recorded 10.25 days in Frieswal heifers and 21 days in Sahiwal Heifers respectively. While, Ahmed et al. (2012) recorded 7-14 days oestrus induction in anoestrus cow after treating with Vitamin AD₃E which was lower than the days recorded in present study. Similarly Bhaskar et al. (2013) recorded oestrus induction 21.17 ± 1.08 days in post partum anoestrus cow after giving Vitamin A and Tonophosphan. Whereas higher days of oestrus induction than the present study were recorded by Kulkarni (1973) reported 28.66 ± 6.7 days required to induce estrus after giving treatment with Tonophosphan + Prepalin in anoestrus heifers. Similarly Kumar et al. (2011) recorded 26.70 ± 4.52 days after treatment with Tonophosphan + Prepalin + Mineral supplements in crossbred anoestrus cow and Markandeya et al. (2002) recorded 75 days in anoestrus Deoni cow after Gynalactin supplementation.

The results pertaining to the GnRH treated Group recorded in the present study are comparable with Thakur and Bhatt (2001) they recorded 23.50 ± 1.98 days of oestrus induction in crossbred heifer. Whereas lesser period than the present study was recorded by Nautiyal et al. (1997), who given inj. GnRH with two dose regimen, 1.5 ml and 2.5 ml and they recorded 3.46 and 2.60 days required to induce estrus for two dose regimens in anoestrus pubertal heifers respectively. While Sirmour et al. (2006) recorded 12.60 ± 3 days of oestrus induction period respectively in crossbred heifers after GnRH treatment. Similarly, for anestrus cows by Mujumdar (1989), Zaghloul et al. (1993), Sonwane et al. (1995) Gupta et al. (2012) and More (2013) they recorded 8 to 14, 3 to 25, 8 to 20 10.16 ± 0.33 and 9.80 days of oestrus induction respectively. However, Kumar et al. (2011) reported mean interval of 28.27 ± 6.46 days in true anoestrus cows for induction of oestrus with GnRH which is higher than the present findings. Markandeya and Bharkad (2004) recorded 21.35 ± 1.98, 23.69 ± 1.69 days in early and late winter season respectively and Bhutani et al. (2009) found 21.50 ± 5.49 days estrus induction in anoestrus buffaloes respectively which was lower than present findings.

In present study, the difference in time interval recorded for onset of oestrus with GnRH and vitamin AD₃E+ Phosphorus treated groups in delayed pubertal crossbred heifer was found to be marginal (24.48 vs. 22.25 days). Thus the present study indicated that GnRH and vitamin AD₃E+ Phosphorus can induce the oestrus
at somewhat similar time interval. However, the difference was much more when both treatment Groups were compared with Clomiphene Citrate treated Group (37.16 days). But if we compare treatment Group I, II, III with Control Group it showed that treated Groups are highly effective in induction of oestrus in delayed pubertal cross bred heifers than Control Group. As in Control Group no heifers had shown oestrus within 60 day of treatment interval.

The time interval recorded in the treatment Groups of present study suggests that GnRH secretion becomes more rapidly pulsatile with the administration of Buserelin-acetate and Clomiphene citrate which results in increased pituitary Gonadotropin (FSH and LH) release. Thus it is opined that GnRH and Clomiphene citrate are effective in up regulation of the hypothalamic-pituitary-gonadal axis.

Administration of Vitamin AD₃E+ Phosphorus in delayed pubertal crossbred heifers suffering from reduced fertility improve their reproductive performance as indicated by resumption of ovarian activity and oestrus induction. It can be recommended to supply livestock with such elements to decrease the incidence of delayed puberty in heifers.

### 4.3 Duration of Oestrus

The delayed pubertal crossbred heifers responded to the treatment of Clomiphene citrate, Vitamin AD₃E + Phosphorus and GnRH Groups were observed and observations regarding duration of oestrus were presented in Table 3.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of animals</th>
<th>Duration of oestrus (hrs)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>20.33 (18 - 23)</td>
<td>2:3</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>22.40 (21 - 23)</td>
<td>2:3</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>21.43 (19 - 23)</td>
<td>2:3</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
The range of duration of oestrus from experimental heifer from Group I was 18 to 23 hrs with a mean of 20.33 hrs In Group II range from 21 to 23 hrs with a mean of 22.40 hrs In Group III range from 19 to 23 hrs with a mean of 21.43 hrs and in Group IV no animal shown oestrus. The present findings were revelled that the duration of oestrus was more in Vitamin AD₃E + Phosphorus Group followed GnRH and Clomiphene Citrate treated Groups.

For Group II the present findings were lower than the values reported by Markandeya et al. (2002) they recorded duration of oestrus 23.04 ± 2.18 hrs in anoestrus cows treated with Gynalactin + Mineral supplement. Whereas, lower values than present study for Group II was recorded by Baruah et al. (1989) recorded 17.90 ± 0.48 hrs duration of oestrus in crossbred heifers after giving beta carotene treatment.

In Group III the present findings of duration of oestrus in hrs were comparable to the observations recorded by Markandeya and Bharkad (2004) reported 21.35 ± 1.98 hrs in postpartum buffaloes treated with Receptal. Whereas higher duration of oestrus hrs than the present findings were observed by Dantre et al. (1998) reported duration of oestrus 23.5 ± 1.89 hrs in crossbred heifers.

On scanning of available literature no references on duration of oestrus in induced oestrus by Clomiphene citrate treated group in delayed pubertal crossbred heifers was found.

From present findings it is concluded that Clomiphene citrate treated Group I heifer showed low duration of oestrus (20.33 hrs) as compared to GnRH treated Group III (21.43 hrs) and then in Vitamin AD₃E + Phosphorus treated (22.40 hrs) Group II.

4.4 Oestrus Intensity Score

Optimal behavioural display of oestrus indicates the level of well-being among dairy cattle. Moreover, full oestrous signals display the female receptivity for mating is in direct relation to spontaneous ovulation, oestrous intensity. This would facilitate proper timing for artificial insemination (AI) and, ultimately, affect reproductive performance. Visual recording is the most accurate method to
determine presence and intensity of oestrous signs. Further, Garcia et al. (2011) showed that the higher the intensity of oestrus, the higher the pregnancy and calving rates achieved (80 and 75 %, respectively). Oestrus of lower intensity is associated with delayed ovulation, reduced preovulatory oestradiol (E<sub>2</sub>) concentration, and poor oocyte quality.

Present study was carried out in delayed pubertal crossbred heifers under field condition in Pune, Nasik, and Ahmednagar region of Maharashtra state. The data pertaining to oestrus sign and oestrus intensity score was recorded and presented in Table 3.

Oestrus behavioural responses of all 17 treated heifers out of 32 heifers in this study were detected after the treatment with Clomiphene citrate, Vitamin AD<sub>3</sub>E + Phosphorus and GnRH. Scoring of oestrus symptoms for observed oestrus was done according to Rao et al. (2012) and reported in Table 3.

Table 3. indicate that all heifers from Group I, II, and III showed oestrus intensity score from 2 to 3 while group IV (control) in which no heifer showed any oestrus sign. Present study showed that in Group I, three animal shows standing oestrus whereas Group II and III only one animal showed standing oestrus respectively. It indicates that the induced heat in treated animal was more pronounced in Group II.

Sirmour et al. (2006) reported 77.40 ± 4.01 oestrus intensity score in crossbred heifers after giving injection Receptal. Ahmed et al. (2012) recorded 3.46 ± 1.33 oestrus intensity score in anoestrus Egyptian cows, on vitamin AD<sub>3</sub>E supplementation. Whereas, Baruah et al. (1989) observed 78.57 % heifers with pronounced oestrus intensity and 21.43 % heifers with weak intensity in anoestrus crossbred heifer on giving beta carotene supplement. The present findings were not comparable to the finding recorded by above workers because they use different scoring methods for oestrus induction than the present study.

In present study Control Group heifers did not show any oestrus this may be due to deficiency of calcium, phosphorus and other vitamin deficiency which impaired the oestrus pattern and oestrus in the heifers. Some animals showed weak oestrus this might be due to the depressed production of steroids hormone in affected animals (Jackson et al., 1981) and may be of β carotene deficiency and this
deficiency is due to acute scarcity of green fodder. Present study reveals that it might be possible that the oestrus behaviour level of heifers may depend on neuroendocrine and individual oestrus behaviour.

4.5. Conception Rate

The delayed pubertal crossbred heifers responded to the treatment of Clomiphene citrate, Vitamin AD₃E + Phosphorus and GnRH Groups were inseminated with frozen thawed semen at 12 hours interval from the onset of oestrus. All the inseminated heifers were examined per rectally 60 days after insemination for diagnosis of pregnancy.

Conception rate during the present study was presented in Table 1. The conception rates from responded heifers were 33.33 % (2/6), 80 % (4/5), 85.71 % (6/7) and 0 % (0/0) respectively in Group I, II, III, and IV.

The present findings in Clomiphene Citrate treated Group were lower than the findings recorded by Purohit and Bishnoi (1993), reported conception rate of 71.42 % in anoestrus Rathi heifers. While Kurien and Madhavan (1985) and More (2013) reported 42.11 and 60 % conception rate in crossbred cow respectively. Whereas, Kodagali (1978) and Kurien and Madhavan (1985) recorded lower conception rate 25 and 30.30 % respectively than the present study in delayed pubertal crossbred heifers. However, Hukeri et al. (1979), Banerjee and Roychoudhary (1989), Kadu and Chede (1992) and Reddy et al. (1994) they recorded 80.00, 75, 84.10 and 87.50 % conception rate respectively in anoestrus buffaloes treated with Clomiphene citrate which was higher than present study. Similarly lower Conception rate than present study was reported by Deen and Tanwar (1988) in anoestrus buffaloes.

In present study the conception rate for Group II was 80 %. The present observation was higher than the observations recorded by Kulkarni et al. (1973) found 36.36 % conception rate in heifers after treatment with Tonophosphan + Prepalin. Similarly Hussain et al. (2009) recorded 71.4 % conception rate in post partum anoestrus cow after supplementation of Agrimin mineral mixture. Kumar et al. (2011) recorded conception rate 40 % in anoestrus cross bred cow in Tonophosphan + Prepalin + Mineral mixture supplementation. Ahmed et al. (2012)
inject Vit. AD$_3$E and recorded 64 % conception rate in anoestrous cows, The higher conception rate than present study was recorded by Mathur et al. (2004) found 100 % conception rate in Frieswal and Sahiwal heifers after treatment with Tonophosphan + Prepalin. Similarly, Markandeya et al. (2002) recorded 100 % conception rate in anoestrous Deoni cows by giving Gynolactine (non hormonal micro mineral preparation with vitamin E).

The conception rate observed in the present study pertaining to GnRH was found higher than the observations recorded by Nautiyal et al. (1997), Dhantrre et al. (1998), Thakur and Bhatt (2001) and Sirmour et al. (2006) they reported conception rate 80.00, 66.66, 66.66 and 40 % in heifers respectively. Whereas lower values of conception rate was recorded by Kumar et al. (2011) Gupta et al. (2012) and More (2013) they recorded 72.73, 80.00 and 83.33 % conception rate respectively in crossbred cows. Similarly Markandeyya and Bharkad (2004) found 75 and 50 % in early and late winter season respectively and Bhutani et al. (2009) recorded 38.46 % conception rate in anoestrous buffaloes. Higher value than present study was recorded by Zaghloul et al. (1993) recorded 100 % conception rate in anoestrous jersey cow. The conception rate recorded pertaining Group III in present findings were found to be similar with Sonwane et al. (1995) they reported 85.71 % conception rate in anoestrous cows treated with Receptal.

From the present study it was observed that the conception rate was higher (85.71 %) in the Group of animals treated with Gonadotropin releasing hormone followed by the Group of animals treated with Vitamin AD$_3$E + Phosphorus (80 %) and Clomiphene citrate (33.33 %). Thus, it is opined that the GnRH is more effective in inducing oestrus and fertility in delayed pubertal cross bred heifers.

4.6 Biochemical Profile

The blood and mineral profile during anoestrous period has a great relevance to future fertility in dairy animals. The presence of low or very low mineral status in blood and the response to specific mineral element may be helpful in the diagnosis of mineral specific disorder. Keeping in view the above facts the study was carried out to assess the effect of certain drugs on biochemical profiles of delayed pubertal heifers.
Biochemical profile of all the delayed pubertal crossbred heifers were studied with regards to serum calcium, serum inorganic phosphorus, total protein, albumin, globulin and glucose before treatment and at the time of oestrus. The estimated biochemical values are presented in Table 4.

**Table 4. Biochemical profile in delayed pubertal crossbred heifers (n=32)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Period of sample collection</th>
<th>Serum calcium (mg/dL)</th>
<th>Serum inorganic phosphorus (mg/dL)</th>
<th>Blood glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n=8)</td>
<td>Before treatment</td>
<td>7.37±0.21</td>
<td>3.75±0.40</td>
<td>58.62±0.71</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>10.02±0.57**</td>
<td>5.82±0.43**</td>
<td>64.52±1.70**</td>
</tr>
<tr>
<td>II (n=8)</td>
<td>Before treatment</td>
<td>10.05±0.25</td>
<td>4.35±0.22</td>
<td>54.75±2.27</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>11.20±0.28**</td>
<td>6.35±0.44**</td>
<td>67.10±2.67**</td>
</tr>
<tr>
<td>III (n=8)</td>
<td>Before treatment</td>
<td>8.24±0.30</td>
<td>3.47±0.20</td>
<td>59.13±2.95</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>10.04±0.55**</td>
<td>6.36±0.44**</td>
<td>68.63±2.26**</td>
</tr>
<tr>
<td>IV (n=8)</td>
<td>Before treatment</td>
<td>7.37±0.32</td>
<td>3.71±0.38</td>
<td>59.62±1.74</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>7.58±0.25 NS</td>
<td>3.99±0.37 NS</td>
<td>58.25±1.84 NS</td>
</tr>
</tbody>
</table>

** significant at 5% level, NS Non Significant

### 4.6.1 Serum calcium

From Table 4, it was found that before treatment serum calcium values for Group I were ranged 6.39 to 8.13 with a mean of 7.37 ± 0.21 mg/dL, for Group II were ranged 9.15 to 11.24 with mean of 10.05 ± 0.25 mg/dL, for Group III were ranged from 7.14 to 9.26 with mean of 8.24 ± 0.30 mg/dL and for control Group it ranged from 6.02 to 8.60 with a mean of 7.37 ± 0.32 mg/dL.

After treatment the serum calcium values for Group I, were ranged between 7.20 to 12.03 with a mean of 10.02 ± 0.57 mg/dL, for Group II were ranged from 10.25 to 12.56 with a mean of 11.20 ± 0.28 mg/dL and for Group III were ranged between 7.80 to 12.00 with a mean of 10.04 ± 0.55 mg/dL respectively. In Group IV (control), no animal shown oestrus during the entire study, therefore the values of serum Calcium were estimated after 60 days of selection and which was as 6.41 to 8.40 with a mean of 7.58 ± 0.25 mg/dL.

From the above data, it was evident that there were increases in the serum calcium values in heifers after the treatment of Clomiphene citrate, inj. of Vitamin
AD_{3}E+ Phosphorus and GnRH. On statistical analysis it was found that there was significantly difference (P<0.05) in the serum calcium value before and after treatment in all Groups except in Control Group there was marginal rise was recorded in serum calcium levels with no statistical significant difference (P<0.05).

The present findings of Group I are in agreement with Purohit and Bishnoi (1993) they found the serum calcium level Before treatment was 10.47 ± 0.904 mg/dL and after treatment the level increased up to 11.30 ± 0.825 mg/dL in responded animals. Similarly Prasad et al. (1984) reported serum calcium level in anoestrus crossbred cow as 9.82 ± 0.36 mg/dL before treatment and 10.05 ± 0.39 mg/dL after treatment with Fertivet.

The present findings in Group II are in agreement with the Singh and Vadnere (1987) and Singh (2006) they reported serum calcium value 9.667 ± 0.25 mg/dl, 8.63 ± 0.31 mg/dL, before treatment and 12.420 ± 0.28 mg/dL, 9.67 ± 0.29 mg/dL after treatment in cross bred anoestrus cows treated with mineral supplement. Similarly Behera et al. (1993) reported significant difference for (P<0.05) between pre treatment (10.01 ± 0.10 mg/dL) and post treatment values (10.27 ± 0.11 mg/dL) in delayed pubertal cross bred heifers treated with Tonophosphphan and Mineral mixture. Whereas Singh et al. (2006) reported 9.36 ± 0.44 mg/dL before treatment and 10.19 ± 0.40 mg/dL after treatment in crossbred cattle treated with Receptal.

The present observation of Group III is comparable with Dantre et al. (1998) they recorded significant difference (P<0.05) before treatment (9.59 ± 0.79) and after treatment (10.39 ± 0.78mg/dL) in serum calcium level in delayed pubertal crossbred heifers and Singh et al. (2006) also reported 9.36 ± 0.44 mg/dL before treatment and 10.19 ± 0.40 mg/dL after treatment in cross bred cattle treated with Receptal.

The present study also reveals that (Annexure - II) in the responding heifers on the day of oestrus there was rise in calcium values than the Calcium values of delayed pubertal anoestrus heifers in Group I, II, and III. The present findings were in accordance with the findings of Bagal and Kadu (1988) and More (2013) in GnRH treated post partum cows. Whereas Dantre et al. (1998) in crossbred heifer, Ahmed et al. (2010) in buffalo heifer and Singh et al. (2006) in crossbred cattle after giving
injection Receptal. The highest level of serum calcium in the cows of different Groups at induced oestrus in the present study might be due to increased level of estrogen which mobilized calcium from different body depots to blood that is necessary for genital tract contraction (Hafez, 1987).

In the non-responded heifers in the present study, the calcium level in the circulation might not have reached optimal level essential for showing oestrus symptoms, as calcium is necessary to sensitize the tubular genitalia for the action of hormones.

4.6.2 Serum inorganic phosphorus

The serum phosphorus values recorded in the delayed pubertal crossbred heifers before treatment and after the treatment are presented in Table 5. The data revealed that the serum inorganic phosphorus level before treatment ranged from 2.30 to 5.82 with mean of 3.75 ± 0.40 mg/dL in Group I. For Group II were ranges from 3.50 to 5.29 with mean of 4.35 ± 0.22 mg/dL. Whereas in Group III were ranges from 2.56 to 4.20 with a mean of 3.47 ± 0.20 mg/dL and for Group IV it range from 2.10 to 5.74 with mean of 3.71 ± 0.38 mg/dL.

After treatment the serum inorganic phosphorus values ranges from 4.26 to 7.96 with mean of 5.82 ± 0.43 mg/dL in Group I. For Group II were ranges from 5.02 to 8.54 with mean of 6.35 ± 0.44 mg/dL. Whereas in Group III were ranges from 4.84 to 7.83 with mean of 6.36 ± 0.44 mg/dL and For Group IV range from 2.73 to 6.01 with mean of 3.99 ± 0.37 mg/dL.

It was observed that phosphorous values after treatment were increased in all treated Group. In except in Control Group there was slight increase in serum phosphorus values at the end of experiment. On statistical analysis there was significant difference (P<0.05) was noted in the post treatment values of serum phosphorus over their pre treatment values in Group I, Group II and Group III heifers. Similarly, Control Group heifers did not showed any statistical difference (P<0.05) in serum phosphorus value after treatment.

In Group I the results of the present study are in agreement with Prasad et al. (1984) reported average serum inorganic phosphorus level in ten anoestrous crossbred cow as 7.33 ± 0.63 mg/dL before treatment and 7.59 ± 0.61 mg/dL after
treatment with tablet Fertivet. While it was partial agreement with Purohit and Bishnoi (1993) as they recorded higher (5.65 ± 0.66 mg/dL) but non-significant serum inorganic phosphorus level in anoestrus cows induced for oestrus with Fertivet over the (5.24 ± 0.89 mg/dL) pre-treatment value.

The present study reveals that after supplementation of Vitamin AD$_3$E and Phosphorus, the inorganic phosphorous values in delayed pubertal crossbred heifers were significantly goes higher (P<0.05) after treatment. The present findings were in agreement with the Singh and Vadnere (1987) they reported 6.22 ± 0.02 mg/dL before treatment and 7.19 ± 0.15 mg/dL after treatment in cross bred anoestrus cows treated with mineral supplement. Similarly Behera et al. (1993) reported significant difference for (P<0.01) between pre treatment (3.03 ± 0.12 mg/dL) and post treatment values (5.26 ± 0.12 mg/dL) in delayed pubertal cross bred heifers treated with Mineral mixture and Tonophosphan and Singh et al. (2006) reported 4.84 ± 0.24 mg/dL before treatment and 8.34 ± 0.16 mg/dL after treatment in cross bred cattle treated with Urimin.

The present findings in Group III are in agreement with Dantre et al. (1998) they recorded that the serum inorganic phosphorus level recorded during post treatment (5.06 ± 0.23 mg/dL) was also found to be significantly higher (P<0.05) than pre- treatment period (4.59 ± 0.24 mg/dL) when the animals were subjected to GnRH treatment. Similarly Singh et al. (2006) also found significant increase in serum phosphorus level (5.03 ± 0.26 mg/dL) before treatment and 6.68 ± 0.40 mg/dL after treatment in cross bred cattle. Significant difference between pre and post treatment stage in Receptal treated animal indicate that GnRH treatment regulate the level of hormone responsible for reproduction and also the level of serum phosphorus by bringing their level to optimum

The present study (Annexure II) also showed that the heifers in oestrus after treatment showed rise in phosphorus value than the phosphorus values of delayed pubertal heifers in Group I, II, and III. The present findings were in accordance with the findings of Chandoliya et al. (1987), Datta et al. (2001) and Yadav et al. (2004). They found significantly higher values of serum inorganic phosphorus during oestrus than during the anoestrus phase.
Significantly elevated concentration of serum inorganic phosphorus obtained on the day of induced oestrus in all the Groups might reflect a concomitant increase of the mineral level with increase in estrogen concentration during oestrus which was responsible for increased phosphorus absorption and retention during reproductive phase.

It was stated that the lower level of calcium and phosphorus may be responsible for delay in puberty as calcium responded mechanisms are involved in steroid bio-synthesis in ovaries. In present study the significant increase in the calcium and inorganic phosphorus level in heifers after the treatment and in induced oestrus after it may be due to supplementation of mineral mixture before the giving treatment or due to fluctuating levels of estrogen in oestrus and the lower levels of serum calcium in delayed pubertal heifers might be due to failure of endocrine system to mobilized the body calcium which leads to reproductive failure. Dutta et al. (2001).

Lazar et al. (1992) during the study on calcium signalling and episodic secretion of GnRH stated that the pulsatility of GnRH could be abolished in Ca\(^{2+}\) deficient medium. It was demonstrated that pulsatile neuropeptide secretion was an intrinsic property of GnRH neuronal networks and was dependent on Ca\(^{2+}\) influx for its maintenance.

The low level of serum calcium and phosphorus in delayed heifers reported in present study under field condition might be due to intake of Calcium and phosphorus deficient feeds. It can be further stressed that the imbalance calcium and phosphorus ration (P<0.01) might have resulted in pituitary gonadal dysfunction and delay in the onset of maturity in crossbred heifers. Commonly available dry roughages, mainly paddy straw contain low level of most of the micronutrient except iron. Phosphorus content in most of the grasses was deficient which might due to the fact that phosphorus contain in soil was deficient as well as high contain of iron in the soil render the phosphorus as insoluble phosphate (Maynard et al., 1979)

On scanning the available literature it was revealed that no studies have been conducted where in the direct relationship of serum Calcium, inorganic Phosphorus with GnRH, Clomiphene citrate and supplementation of vitamin and Phosphorous could be established. Therefore, at this juncture it is difficult to
comment on the mechanism of increasing levels of phosphorus during induced oestrus and after the treatment. Calcium and Phosphorus is an important mineral from reproduction point of view. During the present study serum Calcium and inorganic Phosphorus levels were significant and higher levels of serum calcium and inorganic Phosphorus level at induced oestrus is found which has also been reported by some of the workers (Dantre et al. 1998) draws attention for detailed investigation.

The present findings supports the view that low calcium and inorganic phosphorus levels in blood serum might be responsible for the delay in puberty or anoestrus like condition in cross bred heifers under poor livestock managerial practices.

**4.6.3 Blood glucose**

Table 4. show the data pertaining to the blood glucose before treatment and after treatment. The animal which did not showed oestrus, blood glucose values were estimated after 60 day after completion of treatment from selected heifers.

In the present study the glucose values before treatment for Group I, were range from 56 to 61 with mean of 58.63 ± 0.71 mg/dL. For Group II were ranges from 46 to 65 with mean of 54.75 ± 2.27 mg/dL. In Group III were range from 46 to 73 with mean of 59.13 ± 2.95 mg/dL and for Group IV were ranges from 52 to 70 with mean of 59.62 ± 1.74 mg/dL respectively. Whereas glucose values after treatment for Group I were ranges from 59.10 to 72 with mean of 64.53 ± 1.70 mg/dL. For Group II were ranges from 58 to 78 with mean of 67.10 ± 2.67 mg/dL. In Group III were ranges from 59 to 80 with mean of 68.63 ± 2.26 mg/dL and for Group IV range from 48 to 65 with mean of 58.25 ± 1.84 mg/dL.

From the data it was found that after the treatment there were increases in the glucose values than the values before the treatment in all Groups. However in Group IV marginal decrease was recorded in glucose level after 60 days. On statistical analysis it was found that there was statistically significant difference (P<0.05) in glucose values before and after treatment in all 3 Groups except in Control Group where difference was statistically non significant (P<0.05).
The present findings pertaining to Clomiphene citrate are in non-agreement with Purohit and Bishnoi (1993) they recorded non-significant difference in the levels of serum glucose before treatment (44.27 ± 3.26 mg/dL) and after treatment (46.07 ± 5.73 mg/dL) in the heifers treated with Fertivet. More (2013) recorded non-significant difference in the levels of serum glucose before treatment (54.72 ± 2.76 mg/dL) and after treatment (61.52 ± 3.28 mg/dL) in the post partum anoestrus cows treated with Fertivet.

The present findings pertaining to Group II are in agreement with Kulkarni (1973) who reported glucose level 57.83 ± 7 mg/dL in control group and 64.25 ± 7.33 mg/dL in treated group after Tonophosphan and vitamin A treatment in anoestrus heifers.

The present findings pertaining to GnRH Group are in agreement with Dantre et al. (1998) they recorded significant increase (P<0.05) in the post-treatment mean serum glucose values in the delayed pubertal crossbred heifers when treated with Receptal whereas More (2013) recorded significant difference in the levels of serum glucose before treatment (49.11±1.74mg/dL) and after treatment (60.12±2.17mg/dL) in the post partum anoestrus cows treated with Receptal.

Bagal and Kadu (1988) recorded no significant difference in the blood glucose levels of postpartum cross bred cows treated with GnRH and non-treated cows during first postpartum ovulation and also at detected oestrus. Thus the present findings are not in agreement with the observation of Bagal and Kadu (1988). The disagreement may be due to the differences in the physiological status of the animals since delayed pubertal heifers were included in the present study, whereas Bagal and Kadu (1988) conducted their study on postpartum crossbred cows without any history of anoestrus condition.

Agarwal et al. (1985), Shrivastava and Kharche (1986), Ramkrishna (1997) and Singh and Singh et al. (2005) reported significantly lower serum glucose values in the anoestrus animals than the normal cyclic animals. Similarly in present study the lower glucose values were seen in delayed pubertal heifers than the heifers in induced oestrus.

The blood glucose level influences the pituitary function. The anoestrus or delayed pubertal condition in heifers indicates a low energy status which affects the
follicular development resulting in follicular atresia and anoestrus. In the present investigation, positive correlation was observed between the GnRH, Clomiphene citrate and Vitamin and Phosphorus supplement and blood glucose levels. A previous research has documented the relationship between the GnRH and glucose metabolism. Mc Cann and Hansel (1986) also reported that the aberrant pituitary and luteal functions in fasted heifers were associated with concurrent fasting-induced changes in insulin and glucose metabolism. Similarly secretion of Gonadotropin might have reduced or stopped due to hypothalamic failure to utilized glucose.

In conclusion the delayed pubertal heifers showed lower blood glucose values which indicate lower energy status.

### 4.6.4 Total protein

The total protein, albumin and globulin values recorded in the delayed pubertal crossbred heifer before treatment and at the onset of oestrus are presented in Table 5.

<table>
<thead>
<tr>
<th>Group</th>
<th>Period of sample collection</th>
<th>Serum total protein (gm/dL)</th>
<th>Serum Albumin (gm/dL)</th>
<th>Serum Globulin (gm/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Before treatment</td>
<td>4.89±0.32</td>
<td>2.54±0.24</td>
<td>2.36±0.49</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>6.47±0.58**</td>
<td>3.68±0.19**</td>
<td>2.80±0.61NS</td>
</tr>
<tr>
<td>II</td>
<td>Before treatment</td>
<td>4.67±0.44</td>
<td>3.30±0.36</td>
<td>1.37±0.17</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>6.28±0.38**</td>
<td>4.16±0.18**</td>
<td>2.11±0.26**</td>
</tr>
<tr>
<td>III</td>
<td>Before treatment</td>
<td>4.60±0.29</td>
<td>2.80±0.18</td>
<td>1.81±0.31</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>6.05±0.24**</td>
<td>3.62±0.28**</td>
<td>2.44±0.37NS</td>
</tr>
<tr>
<td>IV</td>
<td>Before treatment</td>
<td>3.31±0.25</td>
<td>2.22±0.19</td>
<td>1.10±0.18</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>3.79±0.48NS</td>
<td>2.46±0.28NS</td>
<td>1.33±0.28NS</td>
</tr>
</tbody>
</table>

** Significant at 5% level, **NS** Non Significant

In the present study the total protein values before treatment for Group I was range from 3.80 to 6.30 with a mean 4.89 ± 0.32 gm/dL. In Group II range from 2.90 to 6.20 with a mean 4.67 ± 0.44 gm/dL. Whereas, in Group III it range from 3.10 to 5.53 with a mean 4.60 ± 0.29 gm/dL and for Group IV range from 2.10 to 4.20 with a mean 3.31 ± 0.25 gm/dL.
In the present study the total protein values after treatment for Group I was range from 3.89 to 8.69 with a mean 6.47 ± 0.58 gm/dL. In Group II range from 5.01 to 7.59 with a mean 6.28 ± 0.38 gm/dL. Whereas, in Group III it range from 4.90 to 6.90 with a mean 6.05 ± 0.24 gm/dL and for Group IV range from 2.13 to 6.60 with a mean 3.79 ± 0.48 gm/dL.

From the data it was found that after the treatment there was increase in the total protein values than the value of before treatment in all Groups of delayed pubertal heifers. In Group IV marginal rise was recorded in serum total protein level after the treatment. On statistical analysis it was found that there was statistically significant difference (P<0.05) in total protein values before and after treatment in all 3 Groups except in Control Group the difference was statistically non significant (P<0.05).

The present findings of Group I were non comparable with the finding reported by Purohit and Bishnoi (1993) they reported no significant difference (P<0.05) in total protein value before (5.99 ± 0.79 gm/dL) and after (6.26 ± 0.725 gm/dL) treatment in anoestrus Rathi heifers after Fertivet treatment.

In present study Group II showed significant increase in the Total protein values. The present findings were comparable to the findings reported by Behera et al. (1993) recorded mean total protein value in pre treated Group was 6.62 ± 0.17 gm/dL and post treated Group it was 7.20 ± 0.12 gm/dL in crossbred heifer after treating with Tonophosphphan + Mineral supplements.

The present findings of total protein for Group III were comparable with the findings recorded by Ahmed et al. (2010) who reported significant difference (P<0.001) in total protein value in anoestrous and cyclic buffalo heifers (4.78 ± 0.11 vs. 5.33 ± 0.09 gm/dL) after giving treatment with Receptal. The present findings of Group III are non accordance with findings of Patel and Dhami et al. (2006), they found non significant (P<0.05) difference for total protein value in treated Group 6.59 ± 0.11 gm/dL and 6.74 ± 0.09 gm/dL in untreated Group in post partum anoestrus cow. Similarly Bagal and Kadu et al. (1988) also reported non significant (P<0.05) difference in total protein value in anoestrous Group (6.74 ± 0.13 vs. 6.59 ± 0.07 gm/dL) and after giving Receptal treatment.
Whereas Khasatiya et al. (2005) recorded statistically significant difference (P<0.01) in total protein value (7.70 ± 0.13 gm/dL) in treated Group when compared with untreated Group (7.01 ± 0.21 gm/dL) after giving Receptal treatment in post partum buffalo. Present findings were in accordance to the findings reported by Khasatiya et al. (2005).

Kavani et al. (1987) emphasized that low level of serum protein influenced the reproductive status in heifers. The lower level of total serum protein may cause deficiency of particular amino acids required for synthesis of various releasing hormone and pituitary hormone causing in turn reproductive disturbances. Protein being the building blocks of the body lack of sufficient protein intake has been regarded as one of the causes of failure of or delay in resumption of oestrous cycle mainly due to retarded development of sex organ.

An optimum level of total protein in blood serum is essential for the expression of oestrus sign in heifers. According to Hafez and Hafez (2000), deficiency of protein intake in cows causes weak expression of oestrus or temporary cessation of oestrus. The low level of serum total protein in anoestrus condition might cause deficiency of certain amino acids required for synthesis of Gonadotropin (Vhora et al., 1995). This could be one of the reason why the delayed pubertal heifers from control Groups were not shown any oestrus signs.

Present study showed that the average value of total protein in the blood serum at oestrus stage was higher than that of delayed pubertal heifers. The present findings were comparable with findings recorded by Singh and Singh (2005) recorded total protein value in delayed pubertal crossbred heifer’s 5.83 ± 0.13 gm/dL and in cyclic heifers at the time of oestrus 6.22 ± 0.11 gm/dL. However Gujar et al. (1990) found 6.34 ± 0.12 gm/dL and 7.59 ± 0.09 gm/dL total protein value in fertile and non fertile heifers respectively. Yadav et al. (2004) also reported higher values of total protein in normal cyclic cows than anoestrus cows (6.78 ± 0.20 vs. 5.16 ± 0.15 gm/dL).

The difference in total protein values noted in the present study might be due to the higher intake of protein and non protein nitrogenous substances associated with higher content of energy density in oestrous heifers. Whereas in anoestrus heifers with mild inflammation and / or ulceration conditions may cause poor
absorption and utilization of protein breakdown products or over hydration resulting into low level of albumin in blood (Hafez and Hafez, 2000).

4.6.5 Albumin

In the present study, the albumin values before treatment for Group I range from 1.50 to 3.61 with a mean of 2.54 ± 0.70 gm/dL. In Group II range from 1.39 to 4.15 with a mean 3.30 ± 1.01 gm/dL. In Group III range from 1.96 to 3.50 with a mean 2.80 ± 0.51 gm/dL, and for Group IV range from 1.60 to 2.97 with a mean 2.22 ± 0.53 gm/dL.

In the present study the total protein values after treatment for Group I range from 3.16 to 4.63 with a mean of 3.68±0.52 gm/dL, Group II range from 3.46 to 5.13 with a mean of 4.16±0.18 gm/dL, Group III range from 2.10 to 4.54 with a mean of 3.62 ± 0.28 gm/dL and for Group IV range from 1.59 to 3.85 with a mean of 2.46 ± 0.28 gm/dL.

On statistical analysis there was significant increase in the albumin values after the treatment in treatment Group I, II and III. Whereas, in Group IV showed non significant (P<0.05) difference for albumin value before and after treatment. Similarly present study also revealed increase in albumin value at the time of oestrus. Similar findings were recorded by Singh and Singh (2005); they recorded albumin value in delayed pubertal crossbred heifer’s 2.11 ± 0.05 gm/dL and in cyclic heifers at the time of oestrus 2.82 ± 0.08 gm/dL.

4.6.6 Globulin

In the present study the globulin values Before treatment for Group I range from 0.19 to 3.99 with a mean of 2.36 ± 0.49 gm/dL, Group II range from 0.74 to 2.05 with a mean of 1.37 ± 0.17 gm/dL, Group III range from 0.46 to 2.73 with a mean of 1.81 ± 0.31 gm/dL and Group IV range from 0.50 to 1.58 with a mean of 1.10 ± 0.18 gm/dL.

In the present study the globulin values after treatment for Group I range from 0.20 to 5.50 with a mean of 2.80 ± 0.61 gm/dL, Group II range from 1.05 to 3.40 with a mean of 2.11 ± 0.26 gm/dL, Group III range from 0.76 to 4.02 with a
mean of 2.44 ± 0.37 gm/dL and Group IV range from 0.18 to 2.75 with a mean of 1.33 ± 0.28 gm/dL.

On statistical analysis there was significant increase in the globulin values after the treatment in treatment Group II. Whereas, Group I, III, and IV showed numerically increased but non significant (P<0.05) difference for globulin value before and after treatment. Similarly present study also revealed increase in globulin value at the time of oestrus in Group I, II and III. The present findings were contradictory to the findings of Singh and Singh (2005) they recorded non significant difference in globulin value in delayed pubertal crossbred heifer’s 3.72 ± 0.10 gm/dL and in cyclic heifers at the time of oestrus were 3.36 ± 0.12 gm/dL.

4.7 Haematological Study

The negative energy balance due to malnutrition in delayed pubertal crossbred heifer is probably the single most important reason for delayed puberty in our country. Hormonal therapies have been recommended to resolve these problems by most workers based on clinical response, but the reports on blood profile monitoring before and after such therapy too little. Therefore, in the present study certain haematological parameter were studied to evaluate clinical response as well as haematological profile before and after the treatment of Clomiphene citrate, Vitamin AD_{3}E + Phosphorus and GnRH in delayed pubertal crossbred heifers.

The average blood profiles of various blood constituents before and after treatment were pooled in Table 6. The values of blood constituent before treatment for Hb, RBC, PCV, MCV, MCH, MCHC, TLC, Neutrophil, Lymphocyte, Eosinophil, Monocyte and platelets for Group I were 10.90 ± 0.27, 7.40 ± 0.52, 33.19 ± 0.77, 47.08 ± 4.95, 12.66 ± 0.39, 32.85 ± 0.35, 13100 ± 423.42, 37 ± 6.14, 53.25 ± 6.28, 7 ± 0.78, 2.75 ± 0.73, 3.21 ± 0.26, respectively, for Group II were 11.24 ± 0.30, 6.20 ± 0.95, 33.98 ± 0.91 64.45 ± 9.02, 12.16 ± 0.5, 33.08 ± 0.23, 14425 ± 638.01, 34.25 ± 7.11, 51.88 ± 6.96, 7.63 ± 1.15, 1.25 ± 0.45, and 3.32 ± 0.26 respectively and in Group III were 10.54 ± 0.35, 7.70 ± 0.22, 32.31 ± 1.38, 42.06 ± 1.64, 13.71 ± 0.67, 32.73 ± 0.64, 20243.75 ± 4884.81, 29.85 ± 3.68, 60.77 ± 3.69, 5.73 ± 0.84, 0.73 ± 0.62, and 5.31 ± 1.22 respectively,
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I Before treatment</th>
<th>Group I After treatment</th>
<th>Group II Before treatment</th>
<th>Group II After treatment</th>
<th>Group III Before treatment</th>
<th>Group III After treatment</th>
<th>Group IV Before treatment</th>
<th>Group IV After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (gm/dL)</td>
<td>10.90±0.27</td>
<td>12.09±0.25*</td>
<td>11.24±0.30</td>
<td>12.96±0.44**</td>
<td>10.54±0.35</td>
<td>11.33±0.45 NS</td>
<td>10.99±0.56</td>
<td>12.20±0.48 NS</td>
</tr>
<tr>
<td>RBC (X 10¹²/L)</td>
<td>7.40±0.52</td>
<td>8.66±0.31 NS</td>
<td>6.20±0.95</td>
<td>9.32±0.37**</td>
<td>7.70±0.22</td>
<td>7.77±0.33 NS</td>
<td>6.90±0.76</td>
<td>8.87±0.38**</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.19±0.77</td>
<td>37.23±0.80**</td>
<td>33.98±0.91</td>
<td>40.48±1.37**</td>
<td>32.31±1.38</td>
<td>35.0±1.47 NS</td>
<td>33.69±1.70</td>
<td>38.59±1.69 NS</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>47.08±4.95</td>
<td>43.18±0.94 NS</td>
<td>64.45±9.02</td>
<td>43.61±1.22**</td>
<td>42.06±1.64</td>
<td>45.19±1.49 NS</td>
<td>54.45±7.85</td>
<td>43.58±1.07 NS</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>12.66±0.39</td>
<td>3.65±0.09**</td>
<td>12.16±0.5</td>
<td>3.82±0.1**</td>
<td>13.71±0.67</td>
<td>3.54±0.18**</td>
<td>12.39±0.37</td>
<td>3.64±0.10**</td>
</tr>
<tr>
<td>MCHC (gm/dL)</td>
<td>32.85±0.35</td>
<td>32.48±0.31 NS</td>
<td>33.08±0.23</td>
<td>32.04±0.39*</td>
<td>32.73±0.64</td>
<td>32.40±0.2 NS</td>
<td>32.61±0.39</td>
<td>31.67±0.41 NS</td>
</tr>
<tr>
<td>TLC (m³)</td>
<td>13100±423.42</td>
<td>13062.5±565.03 NS</td>
<td>14425±638.01</td>
<td>13137.5±1225.76 NS</td>
<td>20243.75±4884.81</td>
<td>11183.3±621.18 NS</td>
<td>13800±830.23</td>
<td>13825±1150.43 NS</td>
</tr>
<tr>
<td>N (%)</td>
<td>37±6.14</td>
<td>26.75±1.69 NS</td>
<td>34.25±7.11</td>
<td>25.38±1.21 NS</td>
<td>29.85±3.68</td>
<td>19.75±1.05**</td>
<td>26.13±5.54</td>
<td>25±0.38 NS</td>
</tr>
<tr>
<td>L (%)</td>
<td>53.25±6.28</td>
<td>65.63±1.95 NS</td>
<td>51.88±6.96</td>
<td>67.63±1.65**</td>
<td>60.77±3.69</td>
<td>72.94±0.92**</td>
<td>66.38±5.59</td>
<td>67.63±0.71 NS</td>
</tr>
<tr>
<td>E (%)</td>
<td>7±0.78</td>
<td>3.88±0.35**</td>
<td>7.63±1.15</td>
<td>3.75±0.41**</td>
<td>5.73±0.84</td>
<td>4.02±0.27 NS</td>
<td>5.88±0.95</td>
<td>3.50±0.63 NS</td>
</tr>
<tr>
<td>M (%)</td>
<td>2.75±0.73</td>
<td>3.75±0.31 NS</td>
<td>1.25±0.45</td>
<td>3.25±0.31**</td>
<td>0.73±0.62</td>
<td>3.29±0.31**</td>
<td>1.88±0.58</td>
<td>3.38±0.18**</td>
</tr>
<tr>
<td>Platelets (lakhs/m³)</td>
<td>3.21±0.26</td>
<td>2.98±0.2 NS</td>
<td>3.32±0.26</td>
<td>3.34±0.29 NS</td>
<td>5.31±1.22</td>
<td>3.72±0.26 NS</td>
<td>4.75±0.97</td>
<td>3.32±0.31 NS</td>
</tr>
</tbody>
</table>

** Significant at 5% level, NS Non Significant
for Group IV 10.99 ± 0.56, 6.90 ± 0.76, 33.69 ± 1.70, 54.45 ± 7.85, 12.39 ± 0.37, 32.61 ± 0.39, 13800 ± 830.23, 26.13 ± 5.54, 66.38 ± 5.59, 5.88 ± 0.95, 1.88 ± 0.58, and 4.75 ± 0.97 respectively.

The values of blood constituent after treatment for Hb, RBC, PCV, MCV, MCH, MCHC, TLC, Neutrophil, Lymphocyte, Eosinophil, Monocyte and platelets for Group I were 12.09 ± 0.25, 8.66 ± 0.31, 37.23 ± 0.80, 43.18 ± 0.94, 3.65 ± 0.09, 32.48 ± 0.31, 13062.5 ± 565.03, 26.75 ± 1.69, 65.63 ± 1.95, 3.88 ± 0.35, 3.75 ± 0.31 and 2.98 ± 0.2 respectively, for Group II were 12.96 ± 0.44, 9.32 ± 0.37, 40.48 ± 1.37, 43.61 ± 1.22, 3.82 ± 0.1, 32.04 ± 0.39, 13137.5 ± 1225.76, 25.38 ± 1.21, 67.63 ± 1.15, 3.75 ± 0.41, 3.25 ± 0.31 and 3.34 ± 0.29 respectively, in Group III were 11.33 ± 0.45, 7.77 ± 0.33, 35.0 ± 1.47, 45.19 ± 1.49, 3.54 ± 0.18, 32.40 ± 0.2, 11183.3 ± 621.18, 19.75 ± 1.05, 72.94 ± 0.92, 4.02 ± 0.27, 3.29 ± 0.31 and 3.72 ± 0.26 respectively, and for Group IV were 12.20 ± 0.48, 8.87 ± 0.38, 38.59 ± 1.69, 43.58 ± 1.07, 3.64 ± 0.10, 31.67 ± 0.41, 13825 ± 1150.43, 25 ± 0.38, 67.63 ± 0.7, 3.50 ± 0.63, 3.38 ± 0.18, and 3.32 ± 0.31 respectively.

The present haematological findings after treatment in Group I showed significant increase in the Hb, PCV, MCH and Monocyte. The present findings for Hb were similar to the findings observed by Shrivastava and Kharche (1986). They were found significant difference (P<0.05) in Hb level i.e. 7.54 gm/dL before treatment and 7.78 gm/dL on the day of oestrus after treatment of Fertivet.

In Group II significant difference was noted in RBC, MCH and Monocyte after supplementation of Vitamin AD₃E + Phosphorus. Pradhan et al. (1995) recorded significant increase in Hb value after giving mineral supplements in post partum anoestrus cow. Present findings were contradictory to the finding reported by Pradhan et al. (1995).

The present haematological finding for Group III after giving treatment with GnRH showed that there were significant increase in the Hb, RBC, MCV, MCH, MCHC, Lymphocyte, Eosinophil and Monocyte. Similar findings were recorded by Abdel – Mohsen et al. (2013) they reported increase in Hb, RBC and MCV after giving GnRH in cow and Victore et al. (2000) reported increase in WBC count after PRID treatment.
The high numerical difference in Hb value in between pre treated and post treated heifers can be concluded that lower level of haemoglobin might be representing some systemic disarrangement due to deficiencies of certain trace minerals which in turn has depressed the physiological reproduction. Though the important of the level of haemoglobin has not been directly implicated in reproductive disorders, yet the decrease in its value is indicative of certain systemic disorder which can indirectly affect the functional activity of the reproductive organ (Pradhan et al., 1995).

Discrepancies in values for various haematological parameters between our finding and findings recorded by other workers were due to differences in sampling interval, methods used, number of cows sampled, and/or degree of metabolic disturbances. Moreover, genetic disturbances between heifers (Mallard et al., 1998) and subtropical condition, management, feeding and changes in hormonal level may be involved (Meglia et al., 2005).

Table 6. also indicates that the delayed pubertal heifers showed decreased erythrocytic parameters (RBC’s count, packed cell volume and haemoglobin contents). At the same time, there were increased TLC and Neutrophils. These findings might be due to absence of estrogen which is responsible for normal cellular and humoral immune response in heifers as reported by Ahmed et al. (1993) and decreased TLC count might be due to migration of leucocytes to be infiltrated in tissue of genital tracks may be another cause, especially in cases associated with bacterial infection and also it was reported that unsuitable agroclimatic condition (Jabbar, 2004).

All the changes after treatment are within normal range. This suggests that GnRH and Clomiphene citrate does not affect the health status and therefore its use is safe for heifers.

In conclusion delayed puberty is a common phenomenon in cattle heifer and is associated with clinicopathological changes; including negative energy balance, anemia, leukocytopenia with oesinophilia and hypoproteinemia. Therefore it can be concluded that sufficient nutrition and proper biosecurity are the main keys for shortening the non productive period of crossbred heifers.
5. SUMMARY AND CONCLUSIONS

The experiment was conducted on 32 delayed pubertal crossbred heifers. These heifers were randomly divided into four different groups with 8 animals in each group. The Group I animals were treated with Clomiphene citrate @ 300 mg daily orally for five days after oral administration of 1% 125 ml of CuSO₄ solution. The Group II animals treated with Inj. Vit AD₃E + Inj. sodium dimethylamino-2-methyl-phenyl-phosphonate-2gm, i/m per week for successive 3 week. Heifers from Group III were treated with single injection of GnRH analogue (Buserelin-acetate an analogue of Gonadotropin releasing hormone) @ 5 ml (0.02 mg). The animals of Group III were kept as control.

Blood samples were collected from all the animals before and after the treatment and at the onset of estrus for hematological and biochemical investigation. However those heifers which not exhibit oestrus, blood were collected after 60 days. The observations were recorded pertaining to the number of animals responded to the treatment and mean time interval required for the onset of estrus. All the responded animals were inseminated with frozen thawed semen at 12 hrs interval after the onset of estrus. After 45-60 days the pregnancy was confirmed by performing per-rectal examination.

6.1 Efficacy of Oestrus Induction

It reveals that 6 out of 8 heifers (75 %) from Group I exhibited oestrus signs, whereas in Group II, 5 out of 8 heifers (62.5 %) exhibited oestrus signs, while in Group III, 7 out of 8 heifers (87.50 %) exhibited oestrus and no heifer from Control Group showed any oestrus sign during the entire period of experiment. GnRH was superior in estrus induction than other groups.
6.2 Oestrus Induction in Days

Mean time interval for Oestrus Induction in Group I heifers were 37.16 days (range from 14 to 50 days), in Group II, 22.20 days (range from range from 7 to 53 days) and in Group III 24.43 days (range from range from 6 to 55 days). However, no heifer from Group IV (Control Group) exhibited oestrus. Estrus induction days required were lower in Group II than the group I and group III.

6.3 Duration of Oestrus

The range of duration of oestrus from experimental heifer from Group I was 18 to 23 hrs with a mean of 20.33 hrs. In Group II range from 21 to 23 hrs with a mean of 22.40 hrs. In Group III range from 19 to 23 hrs with a mean of 21.43 hrs and in Group IV no animal shown oestrus. Duration of estrus was shorter in Clomiphene citrate treated heifers than the GnRH and Inj. Vit AD₃E and phosphorus treated heifers.

6.4 Oestrus Intensity Score

All heifers from Group I, II, and III showed oestrus intensity score from 2 to 3 except Group IV (control) in which no heifer showed any oestrus sign. Present study showed that in Group I, three animal shows standing oestrus where as Group II and III only one animal showed standing oestrus respectively. It indicates that the induced heat in treated animal was more pronounced in Group II.

6.5. Conception Rate

The conception rates from responded heifers were 33.33 % (2/6), 80 % (4/5), 85.71 % (6/7) and 0 % (0/0) respectively from Group I, II, III and IV. Conception rate was more in GnRH treated heifers.
6.6 Biochemical Profile

6.6.1 Mean serum calcium and inorganic phosphorus

In biochemical profile of the delayed pubertal crossbred heifers the mean serum calcium and inorganic phosphorus level before treatment were 7.37 ± 0.21, 3.35 ± 0.40 mg/dL in Group I, 10.05 ± 0.25, 4.35 ± 0.22 mg/dL in Group II, 8.24 ± 0.30, 3.47 ± 0.20 mg/dL in Group III and 7.37 mg/dL 0.32, 3.71 mg/dL0.38 in Group IV respectively. After treatment the values were 10.02 ± 0.57, 5.82 ± 0.43 mg/dL in Group I, 11.20 ± 0.28, 6.35 ± 0.44 mg/dL in Group II, 10.04 ± 0.55, 6.36 ± 0.44 mg/dL in Group III and 7.58 ± 0.25, 3.99 ± 0.37 mg/dL in Group IV respectively. On statistical analysis it was found that there was significantly difference (P<0.05) in the serum calcium and inorganic phosphorus value before and after treatment in all Groups except in Control Group there was marginal rise was recorded in serum calcium levels with no statistical significant difference (P<0.05).

6.6.2 Mean total protein, albumin and globulin

Mean total protein, albumin and globulin values before treatment were 4.89 ± 0.32, 2.54 ± 0.24, 2.36 ± 0.49 in Group I, 4.67 ± 0.44, 3.30 ± 0.36, 1.37 ± 0.17 in Group II, 4.60 ± 0.29, 2.80 ± 0.18, 1.81 ± 0.31 in Group III and 3.31 ± 0.25, 2.22 ± 0.19, 1.10 ± 0.18 in Group IV respectively. After treatment the values were 6.47 ± 0.58, 3.68 ± 0.17, 2.80 ± 0.61 in Group I, 6.28 ± 0.38, 4.16 ± 0.18, 2.11 ± 0.26 in Group II, 6.05 ± 0.24, 3.62 ± 0.28, 2.44 ± 0.37 in Group III and 3.79 ± 0.478, 2.46 ± 0.28, 1.33 ± 0.28 in Group IV respectively. On statistical analysis it was found that there was significantly difference (P<0.05) in the total protein, albumin and globulin value before and after treatment in all Groups except in Control Group and globulin value of IIIrd Group.
6.6.3 Serum glucose

The mean values of serum glucose observed before treatment in Group I, Group II, Group III and Group IV were 58.62 ± 0.71, 54.75 ± 2.27, 59.13 ± 2.95 and 59.62 ± 1.74 mg/dL respectively. These values increased after treatment and were found to be 64.52 ± 1.70, 67.10 ± 2.67, 68.63 ± 2.26 and 58.25 ± 1.84 ml/dL in respective groups. Significant (P<0.05) difference was observed in Group I, II and III animals in respect of serum glucose while except control Group.

6.6.4 Haematological finding

Present haematological finding after treatment in Group I showed that there were significant increase in the Hb, PCV, MCH and Monocyte, in Group II significant difference was noted in RBC, MCH and Monocyte after supplementation of Vitamin AD₃E + Phosphorus. For Group III after giving treatment with GnRH showed that there were significant increase in the Hb, RBC, MCV, MHC, MCHC, Lymphocyte, Eosinophil and Monocyte.

CONCLUSIONS:

1. The mean time interval for induction of oestrus is lower in Vitamin AD₃E+ Phosphorus supplementation as compared to GnRH and Clomiphene citrate treatment Group.
2. Clomiphene citrate treated Group heifer show reduced duration of oestrus as compared to GnRH treated Group, followed by Vitamin AD₃E + Phosphorus Group than control Group.
3. Low energy, anaemia, hypoproteinemia, leukocytosis, eosinophilia, hypocalcaemia and hypo phosphatemia may be the possible etiological factors for delayed puberty in heifers.
4. The biochemical parameters like Ca, P, total protein, albumin, globulin and glucose are increased to normal range on the day of oestrus, after treatment of GnRH, Clomiphene citrate and supplementation of AD₃E + Phosphorus.
5. Treatment of Clomiphene citrate, Vitamin AD₃E+ Phosphorus and GnRH in delayed pubertal crossbred heifers with sufficient nutrition may
decrease the incidence of delayed puberty and thus plays a key role for shortening the non reproductive period of crossbred heifers

6. Clomiphene citrate, Vitamin AD₃E and GnRH treatments are effective in induction of oestrus in delayed pubertal heifers. However GnRH treatment is superior in inducing oestrus with satisfactory conception rate.


Annexure –I

Detail Record of Oestrus Induction Period in Day, Duration Of Oestrus (Hrs), Intensity of Oestrus in All Treated Groups.

Annexure –I a) Detail record of Oestrus induction period in day, Duration of Oestrus (hrs), Intensity of Oestrus in all treated Group I.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oestrus induction period in day</th>
<th>Duration of Oestrus (hrs)</th>
<th>Intensity of Oestrus (SCORE)</th>
<th>Pregnant /non Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50</td>
<td>19</td>
<td>3</td>
<td>Non Pregnant</td>
</tr>
<tr>
<td>2.</td>
<td>45</td>
<td>19</td>
<td>2</td>
<td>Non Pregnant</td>
</tr>
<tr>
<td>3.</td>
<td>44</td>
<td>18</td>
<td>2</td>
<td>Pregnant</td>
</tr>
<tr>
<td>4.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>23</td>
<td>22</td>
<td>3</td>
<td>Pregnant</td>
</tr>
<tr>
<td>7.</td>
<td>14</td>
<td>21</td>
<td>3</td>
<td>Non Pregnant</td>
</tr>
<tr>
<td>8.</td>
<td>47</td>
<td>23</td>
<td>2</td>
<td>Non Pregnant</td>
</tr>
</tbody>
</table>

Annexure –I b) Detail record of Oestrus induction period in day, Duration of Oestrus (hrs), Intensity of Oestrus in all treated Group II.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oestrus induction period in day</th>
<th>Duration of oestrus (hrs)</th>
<th>Intensity of oestrus (SCORE)</th>
<th>Pregnant /non Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>53</td>
<td>23</td>
<td>2</td>
<td>Pregnant</td>
</tr>
<tr>
<td>2.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Non Pregnant</td>
</tr>
<tr>
<td>3.</td>
<td>7</td>
<td>23</td>
<td>3</td>
<td>Pregnant</td>
</tr>
<tr>
<td>4.</td>
<td>5</td>
<td>22</td>
<td>2</td>
<td>Pregnant</td>
</tr>
<tr>
<td>5.</td>
<td>22</td>
<td>23</td>
<td>2</td>
<td>Non Pregnant</td>
</tr>
<tr>
<td>6.</td>
<td>24</td>
<td>21</td>
<td>2</td>
<td>Pregnant</td>
</tr>
<tr>
<td>7.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Annexure –I c) Detail record of Oestrus induction period in day, Duration of oestrus (hrs), Intensity of oestrus in all treated Group III.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oestrus induction period in day</th>
<th>Duration of oestrus (hrs)</th>
<th>Intensity of oestrus (SCORE)</th>
<th>Pregnant /non Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>24</td>
<td>22</td>
<td>2</td>
<td>Pregnant</td>
</tr>
<tr>
<td>2.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Non Pregnant</td>
</tr>
<tr>
<td>3.</td>
<td>55</td>
<td>21</td>
<td>2</td>
<td>Pregnant</td>
</tr>
<tr>
<td>4.</td>
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<td>Pregnant</td>
</tr>
<tr>
<td>5.</td>
<td>6</td>
<td>22</td>
<td>2</td>
<td>Pregnant</td>
</tr>
<tr>
<td>6.</td>
<td>31</td>
<td>22</td>
<td>2</td>
<td>Pregnant</td>
</tr>
<tr>
<td>7.</td>
<td>15</td>
<td>19</td>
<td>3</td>
<td>Pregnant</td>
</tr>
<tr>
<td>8.</td>
<td>23</td>
<td>21</td>
<td>2</td>
<td>Non Pregnant</td>
</tr>
</tbody>
</table>

*These animal not included in the study because they showed estrus after the completion of experimental days i.e. 60 days*

Annexure –I d) Detail record of Oestrus induction period in day, Duration of oestrus (hrs), Intensity of oestrus in all treated Group IV.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oestrus induction period in day</th>
<th>Duration of oestrus (hrs)</th>
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### Annexure II

**Biochemical parameters before, after and on the day of oestrus in Group I.**

#### Annexure II a) Biochemical parameters before, after and on the day of oestrus in Group I.

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*Blood was collected on the day of estrus in heifers
Annexure II b) Biochemical parameters before, after and on the day of oestrus in Group II.

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*blood was collected on the day of oestrus in heifers
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*Blood was collected on the day of oestrus in heifers*
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### Annexure -III

Haematological parameter before, after and on the day of oestrus in all treated heifers.

Annexure III a) Haematological parameter before, after and on the day of oestrus in all treated heifers.

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Annexure III b) Haematological parameter before, after and on the day of oestrus in all treated heifers Group II

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*blood was collected on the day of oestrus in heifers
Annexure III b) Haematological parameter before, after and on the day of oestrus in all treated heifers in Group III

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*blood was collected on the day of oestrus in heifers
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# THESIS ABSTRACT

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<tr>
<th>1</th>
<th>Title of thesis</th>
<th>THERAPEUTIC MANAGEMENT OF DELAYED PUBERTY IN CROSSBRED HEIFERS.</th>
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<tr>
<td>2</td>
<td>Full Name of Student</td>
<td>NAGARE SACHIN BHAGWAN</td>
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</table>
| 3 | Name & Address of Advisor/ Guide | Dr. R. J. CHAUDHARI  
Assistant Professor  
Department of Animal Reproduction, Gynaecology and Obstetrics. Bombay Veterinary College, Parel, Mumbai-12. |
| 4 | Degree to be awarded | MASTER OF VETERINARY SCIENCE (M.V.Sc) |
| 5 | Year of award of degree | 2014 |
| 6 | Major subject | ANIMAL REPRODUCTION, GYNAECOLOGY & OBSTETRICS. |
| 7 | Total number of pages in the thesis | 97 |
| 8 | Number of words in the thesis abstract | 631 |
| 9 | Signature of student | |
| 10 | Signature, Name & Address of forwarding authority (HOD / SH) | Dr. S. R. Chinchkar  
Professor and HOD, Department of Animal Reproduction Gynaecology and Obstetrics  
Bombay Veterinary College, Parel, Mumbai - 40012 |
| 11 | Signature of associate Dean | |
ABSTRACT

Delayed puberty is one of the major reproductive problems in dairy industry. Therefore the present study was undertaken to evaluate the efficacy of Clomiphene citrate, GnRH and combination of Vit. AD3E + Phosphorus in 32 delayed pubertal crossbred heifers. All the experimental delayed pubertal heifers were randomly divided into four groups of eight heifers each. Group I heifers treated with Clomiphene citrate @ 300 mg for 5 days by oral administration, Group II heifers treated with Inj. Vit. AD3E + Inj. Sodium dimethylamino-2-methyl-phenyl-phosphonate-2gm, i/m per week for successive 3 weeks, Group III heifers treated with 5 ml GnRH i/m (0.02 mg) and Group IV heifers were kept as control. The biochemical and haematological profile of all heifers was studied at onset of oestrus, before and after treatment.

In present study the heifers treated with Clomiphene citrate and Vit. AD3E + Phosphorus showed 75.00 and 62.50% oestrus induction rate and 80 and 33.33% conception rate respectively. The data revealed that the rate of induction of oestrus (87.50%) and conception rate (85.71%) was more in heifers treated with GnRH than the other treatment. The mean time interval recorded for onset of estrus and duration of oestrus was 37.16 days, 20.33 hrs in Group I, 22.20 days, 22.40 hrs in Group II and 24.43 days, 21.43 hrs in Group III respectively. No heifers exhibited oestrus from control group.

Haematological and biochemical constituent of blood have great diagnostic value in the clinical practice. Keeping in view these facts an attempt was made to evaluate the difference if any, in both the profiles. The mean serum calcium and inorganic phosphorus level before treatment were 7.37±0.21, 3.75±0.40 mg/dL in Group I, 10.05±0.25, 4.35±0.22 mg/dL in Group II, 8.24±0.30, 3.47±0.20 mg/dL in Group III and 7.37±0.32, 3.71±0.38 mg/dL in Group IV respectively. After treatment the values were 10.02±0.57, 5.82±0.43 mg/dL in Group I, 11.20±0.28, 6.35±0.44 mg/dL in Group II, 10.04±0.55, 6.36±0.44 mg/dL in Group III and 7.58±0.25, 3.99±0.37 mg/dL in Group IV respectively. After treatment the values were 4.89±0.32, 2.54±0.24, 2.36±0.49 in Group I, 4.67±0.44, 3.30±0.36, 1.37±0.17 in Group II, 4.60±0.29, 2.80±0.18, 1.81±0.31 in Group III and 3.31±0.25, 2.22±0.19, 1.10±0.18 in Group IV respectively. After
treatment the values were 6.47±0.58, 3.68±0.17, and 2.80±0.61 in Group I, 6.28±0.38, 4.16±0.18, and 2.11±0.26 in Group II, 6.05±0.24, 3.62±0.28, 2.44±0.37 in Group III and 3.79±0.478, 2.46±0.28, 1.33±0.28 in Group IV respectively. While the mean values of serum glucose observed before treatment in Group I, Group II, Group III and Group IV were 58.62±0.71, 54.75±2.27, 59.13±2.95 and 59.62±1.74 mg/dL respectively. These values increased after treatment and were found to be 64.52±1.70, 67.10±2.67, and 68.63±2.26 and 58.25±1.84 ml/dL in respective groups. Before and after treatment significant (P<0.05) difference was observed in Group I, II and III animals in respect of serum Ca, P, Total Protein, Albumin and Glucose respectively while globulin value are significant (P<0.05) in Group II only.

The present haematological finding after treatment showed that there were significant increase in the Hb, PCV, MCH and Monocyte in Group I. Whereas in RBC, MCH and Monocyte for Group II and in Hb, RBC, MCV, MHC, MCHC, Lymphocyte, Eosinophil and Monocyte for Group III. Data showed that all the haematological changes after treatment are within normal range.

In conclusion, delayed puberty is a common phenomenon in cattle heifers and is associated with clinicopathological changes like lower energy, anemia, hypoproteinemia, leukocytosis, eosinophilia, hypocalcaemia and hypophosphatemia. Proper nutrition, biosecurity and administration of Vitamin AD₃E⁺ Phosphorus in delayed pubertal crossbred heifers may decrease the incidence of delayed puberty and thus plays a major role in shortening the non productive period of crossbred heifers. It also concluded that Clomiphene citrate and Vit. AD₃E⁺Phosphorus treatments are effective in inducing estrus in delayed pubertal heifers; however GnRH is more superior in inducing oestrus and conception rate.
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<td>डॉ. एस.आर. विचनकर, प्राध्यापक, विभाग प्रमुख, पशुप्रजनन, मादीरोग व प्रसुतीशास्त्र विभाग, मुंबई पशुवेद्यकीय महाविद्यालय, परज, मुंबई — 12.</td>
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प्रबंध सारांश

उशीरा माजावर येणान्या संकरित काळवडीमध्ये माजावर उपचारात्मक व्यवस्थापन करणे

उशीरा वयात येणारी जनावरे ही दुःख व्यवसायातील प्रमुख समस्या आहे, ग्हणून सदरचा संशोधन प्रकळ्य हाती घेण्यात आला. सदर प्रकळ्याच्या मध्ये क्लोमीफेन सायट्रेट, जीवनसत्त्व अ, ड़, इ+, फॉंसफरस आणि संप्रेक्षण जी.एन.आर. एच. यांचा वापर प्रभावीपणे उशीरा वयात येणान्या संकरित काळवडीमध्ये करण्यात आला. सदर प्रकळ्यामध्ये 32 संकरित योग्य वेळेवर माजावर न आलेल्या काळवडीचे चार गटात समप्रमाणे (n = 8) विभागणी करण्यात आली. पहिल्या गटातील काळवडीना 1 टक्के कॉपर सल्फेटचे 125 मि.ली. द्रापण पाणित्यानंतर क्लोमीफेन सायट्रेट 300 मि.ली.ग्रेम या प्रमाणे गोष्ट 5 दिवस दिले. दुसर्या गटातील काळवडीना अ,ड,ई जीवनसत्त्व आणि स्फुर्दे इंजेक्शन दर आठवड्यात एकदा स्नायूत असे तीन आठवडे देण्यात आले. त्याच प्रमाणे तिसर्या गटातील काळवडीना 5 मि.ली. जी.एन. आर.एच. संप्रेक्षणाचे इंजेक्शन देण्यात आले आणि चौथ्या गटात गटात आणि कन्नाचे उपचार केला नाही. या दरम्यान उपचार करण्याची आणि उपचार केल्यानंतर 60 दिवसांपर्यंत माजावर येणान्या सर्व काळवडीच्या रक्त घटकांवी तपासणी करण्यात आली. व ज्या कालवडी माजावर नाही आल्या त्याच्या रक्त घटकांच्या 60 व्या दिवशी अभ्यास करण्यात आला.
संशोधनांतः असे दिसून आले की, पहिल्या आणि दुसःन्या गटातील अनुक्रमे 75 तक्यांक आणि 62.50 तक्यांक काळवडी माजावर आल्या. तसेच अनुक्रमे 80 तक्यांक आणि 33.33 तक्यांक काळवडीत गर्भधारण मालकी. त्याच प्रमाणे तिसःन्या गटातील जी.एन.आर.एच. संप्रेक दिलेल्या 87.50 तक्यांक काळवडीत माजावरी लक्षणे दिसून आल्यात आणि त्यात तालाब 85.71 तक्यांक काळवडी मध्ये गर्भधारण मालकी. पहिल्या, दुसःन्या आणि तिसःन्या गटातील काळवडीनास उपचारांनंतर अनुक्रमे 37.16, 22.20 आणि 24.43 दिवस इतका माजावर काळावधी लागला. तसेच अनुक्रमे 20.33, 22.40 आणि 21.43 तास माज दिसून आला. चौथा गटातील कोणतीही काळवड माजावर आली नाही.

रक्त घटकांचा तपास हा निदानासाठी खूप महत्वाचा मानला जातो. हे लक्षण घेतल्या तर संशोधनात हिमेंटोलोजिकल आणि बायोकेमिकल रक्त घटकांचा अभ्यास करण्यात आला. या अभ्यासात असे दिसून आले की, पहिल्या, दुसःन्या आणि तिसःन्या गटामधील काळवडीच्या रक्तात उपचारापूर्वी अनुक्रमे 7.33 ± 0.21, 3.75 ± 0.40 मी.ग्रॅंजी/डी.एल 10.05 ± 0.25, 4.35 ± 0.22 मी.ग्रॅंजी/डी.एल, तिसःन्या 8.24 ± 0.30, 3.47 ± 0.20 मी. ग्रॅंजी/डी.एल आणि 7.37 ± 0.32, 3.71 ± 0.38 इतकी व उपचारांनंतर अनुक्रमे 10.02 0.5, 5.82 0.43 पहिल्या 11.20 ± 0.28, 6.35 ± 0.44 मी. ग्रॅंजी/डी.एल दुसःन्या 10.4 ± 0.55, 6.36 ± 0.44 तिसःन्या व 7.58 ± 0.25, 3.
99 ± 0.37 मी.ग्र./डी.एल कैंस्टीयम व फॉस्फरसची अनुक्रमे इतकी चौथ्या गटातील कालवडीमध्ये आढळून आली.

तसेच प्रथीने, अल्बूमीन, ग्लोबॉलीनची मात्रा संशोधनानाशी 4.89 ± 0.
32, 2.54 ± 0.24, 2.36 ± 0.49 पहील्या 4.67 ± 0.44, 3.30 ± 0.36, 1.37 ±
0.17 दुसर्या, 4.60 ± 0.29, 2.80 ± 0.18, 1.81 ± 0.31 तिसर्या आणि 3.31
± 0.25, 2.22 ± 0.19, 1.10 ± 0.18 इतकी व उपचारा नंतर अनुक्रमे 6.47 ±
0.58, 3.68 ± 0.19, 2.80 ± 0.61 पहील्या गटात, 6.28 ± 0.38, 4.16 ± 0.
18, 2.11 ± 0.26 दुसर्या गटात, 6.05 ± 0.26, 3.62 ± 0.28, 2.44 ± 0.37
तिसर्या गटात आणि 3.79 ± 0.48, 2.46 ± 0.28, 1.33 ± 0.28 इतकी चौथ्या
गटात आढळून आली या नंतर रक्तातील ग्लूकोजची मात्रा संशोधनापूर्वी 58.
62 ± 0.71, 54.75 ± 2.27, 59.13 ± 2.95 आणि 59.62 ± 1.74 मी.ग्र./डी.
एल इतकी अनुक्रमे पहील्या, दुसर्या, तिसर्या आणि चौथ्या गटात व
उपचारानंतर 64.52 ± 1.70, 67.10 ± 2.67 आणि 68.63 ± 2.26 आणि 58.
25 ±1.84 मी.ग्र./डी.एल इतकी अनुक्रमे दिसून आली. वरिष्ठ रक्तातील
चाचणीपूर्व असे दिसून आले की कैंस्टीयम, फॉस्फरक, प्रथीने, अल्बूमीन,
ग्लूकोज यांची अनुक्रमे पहील्या, दुसर्या, तिसर्या गटातील कालवडीमध्ये
उपचारानंतर लक्षणीय वाढ दिसून आली. तसेच ग्लोबॉलीन या घटकाची
कसलीही वाढ दिसून आली नाही. परंतु ही वाढ रक्त घटकाच्या सामान्य
पातकीच्या प्रमाणात दिसून आली.
हिमेंटोलॉजिकल रक्त घटकांचा अभ्यास केला असता फहीम्या
गटातील कालबड़ीच्या रक्तात उपचारानंतर हिमोग्लोबीन, पी.सी.की., एम.
सी.एच. आणि मोनोसाईट मध्ये लक्षणीय वाढ दिसून आलेली. तसेच लालपेशी,
एम.सी.एच. व मोनोसाईट यांची दुसर्या गटातील व हिमोग्लोबीन, आर.बी.
सी., एम.सी.की., एम.सी.एच., एम.सी.एच.सी. व ब्युक्साईट यांची तिसर्या
गटातील कालबड़ीच्या रक्तात लक्षणीय वाढ दिसून आलेली, व ती सामान्य
पातळीत असल्याचे आढळून आले.

संशोधनांती असे निदर्शनास आले की, लोह, उर्जा, अशक्तपणा,
प्रथीने, क्षार व जीवनसत्ते यांची कमतरता कालबड़ी योग्य व्यायाम माजावर न
षेण्यास कारकीभूत ठरले. संतुलित आहार, निरोगी वातावरण, जीवनसत्ते व
क्षार यांचा आहारातील वापर करून कालबड्डीमधील उशिरा व्यायात षेण्याचे
प्रमाण कमी होवू शकते. सदरील संशोधनांती असा निष्कर्ष कादू शकतो
की, उशिरा व्यायात षेण्याचा कालबड्डीमध्ये क्लोमीफेन सायट्रेट, जीवनसत्ते
अ,ड,ई + फोर्सफर्स व संप्रेक्ष जी.एन.ए.एच. एच. यांचा वापर करून माजावर
नियमन करू शकतो तथापि जी.एन.ए.एच. संप्रेक्षकाचा वापर नियमित माज
सुरु होण्यासाठी व गर्भधारणाचा दर उत्कृष्ट प्रकारे मिळविण्या करता
उपयुक्त ठरू शकतो.
VITA

The author, Sachin Nagare, was born on 12th June, 1989 in Pune, Maharashtra. He passed 10th from Narayandas Ramdas Highschool, Indapur in 2005 and 12th from Sadashivrao Mane Vidyalaya, Akluj in 2007.

His ambitious dream of being a veterinarian made him to join Nagpur Veterinary College, from where he passed out with flying colours in 2012. The same year, he joined Bombay Veterinary College for Masters Degree in Animal Reproduction Gynaecology and Obstetrics. He has published a paper during his post graduation days.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>Hb (gm/dL)</td>
<td>10.90±0.27</td>
<td>12.09±0.25*</td>
<td>11.24±0.30</td>
<td>12.96±0.44**</td>
<td>10.54±0.35</td>
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<tr>
<td>RBC (X 10^{12}/L)</td>
<td>7.40±0.52</td>
<td>8.66±0.31NS</td>
<td>6.20±0.95</td>
<td>9.32±0.37**</td>
<td>7.70±0.22</td>
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<tr>
<td>PCV (%)</td>
<td>33.19±0.77</td>
<td>37.23±0.80**</td>
<td>33.98±0.91</td>
<td>40.48±1.37**</td>
<td>32.31±1.38</td>
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<tr>
<td>MCV (FL)</td>
<td>47.08±4.95</td>
<td>43.18±0.94NS</td>
<td>64.45±9.02</td>
<td>43.61±1.22**</td>
<td>42.06±1.64</td>
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<tr>
<td>MCHC (%)</td>
<td>32.85±0.35</td>
<td>32.48±0.31NS</td>
<td>33.08±0.23</td>
<td>32.04±0.39*</td>
<td>32.73±0.64</td>
</tr>
<tr>
<td>TLC(/m^3)</td>
<td>13100±423.42</td>
<td>13062.5±565.03NS</td>
<td>14425±638.01</td>
<td>13137.5±1225.76NS</td>
<td>20243.75±4884.81</td>
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<tr>
<td>N (%)</td>
<td>37±6.14</td>
<td>26.75±1.69NS</td>
<td>34.25±7.11</td>
<td>25.38±1.21NS</td>
<td>29.85±3.68</td>
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<tr>
<td>L (%)</td>
<td>53.25±6.28</td>
<td>65.63±1.95NS</td>
<td>51.88±6.96</td>
<td>67.63±1.15**</td>
<td>60.77±3.69</td>
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<tr>
<td>E (%)</td>
<td>7±0.78</td>
<td>3.88±0.35**</td>
<td>7.63±1.15</td>
<td>3.75±0.41**</td>
<td>5.73±0.84</td>
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<tr>
<td>M (%)</td>
<td>2.75±0.73</td>
<td>3.75±0.31NS</td>
<td>1.25±0.45</td>
<td>3.25±0.31**</td>
<td>0.73±0.62</td>
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<tr>
<td>Platelets (lakhs/m^3)</td>
<td>3.21±0.26</td>
<td>2.98±0.2NS</td>
<td>3.32±0.26</td>
<td>3.34±0.29NS</td>
<td>5.31±1.22</td>
</tr>
</tbody>
</table>

** Significant at 5% level,  NS Non Significant